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Micro-environmental Influences on Skin Homeostasis

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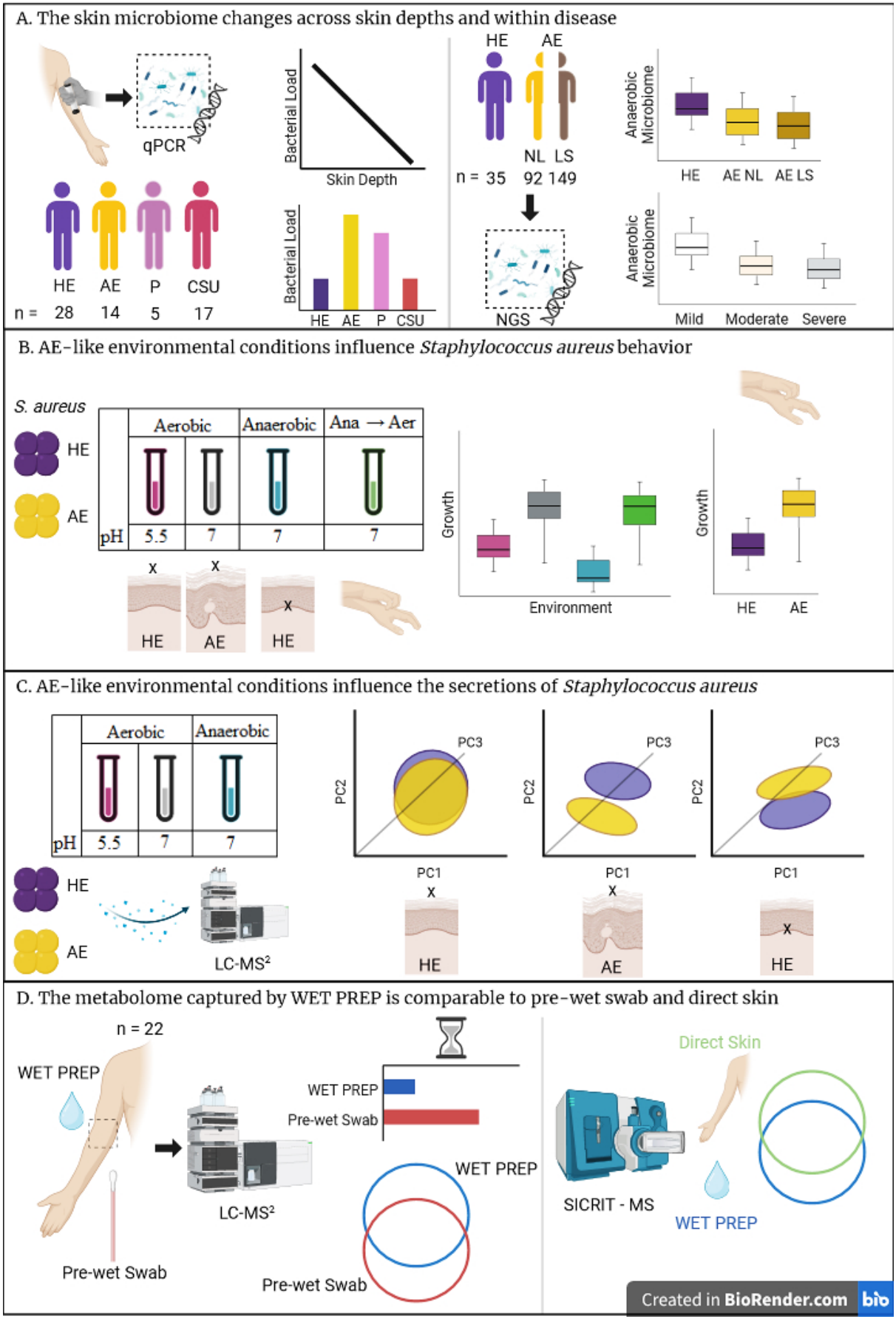
Graphical Abstract



Abstract

Background: Atopic eczema (AE) is an inflammatory skin disorder affecting approximately 20% of children worldwide, with early onset leading to later development of asthma and allergies. Genetic and environmental factors have been shown to contribute to the development of AE. On a micro-environmental level, the skin pH is increased in AE, micro-wounds - caused by scratching - change the availability of oxygen within the skin, and there is an overgrowth of *S. aureus* in AE. Currently, a large proportion of research focuses on how the microbiome changes in AE and how *S. aureus* impacts the immune system in AE. Although the skin is a multi-layered organ, microbiome studies often focus on the skin's surface and do not consider the absolute bacterial abundance but instead only the compositional changes. Also, many immunological studies stimulate the skin with *S. aureus,* either in the epidermis or the dermis, without knowing the actual localization of *S. aureus* within diseased skin. Bacteria are found in the deeper layers of the skin, and there is an oxygen gradient within the skin which can encourage anaerobic bacteria to grow. The micro-wounds in AE would subsequently cause a loss of this oxygen gradient. The anaerobic microbiome is reduced in AE lesional skin. Still, it is currently unknown whether this reduction is specific to lesional skin and its relationship to the severity of AE. In general, there is not one particular type of *S. aureus* but multiple strains that differ in host immunological stimulation and resistance to antibiotics. During an AE flare, one clonal type is found on the skin. Also, a proportion of the healthy population can carry *S. aureus* without developing AE, suggesting healthy and AE strain differentiation. *S. aureus* can influence the skin through small molecule, i.e. metabolite, secretions, and there have yet to be studies connecting *S. aureus's* secretions to the skin metabolome. Although there are various methods for measuring the skin metabolome, the myriad of ways varies in invasiveness with the current non-invasive method, pre-wet swabs, requiring a week of preparation before sampling, making it unfeasible for translation into the clinical setting.

Hypothesis and Aims: This thesis aims to explore the impacts of the micro-environment (the microbiome, oxygen, pH, and *S. aureus*) on skin homeostasis and to create a method to determine skin health through its metabolite signature. ***(A1)*** I hypothesize that the bacterial load differs across the skin layers and within skin disease. As compositional data has shown *S. aureus* to be overgrown in AE; ***(A2)*** I presume it is present in higher absolute abundance within AE. In addition, because bacteria are found across the skin layers, and there is a differential oxygen gradient across the skin, ***(A3)*** there should also be an anaerobic microbiome within the skin. AE skin has micro-wounds and a decrease of structural proteins like tight junctions and

filaggrin. This would hypothetically cause a loss of the skin's oxygen gradient and, consequently, ***(A4)*** a loss of the anaerobic microbiome in both lesional and non-lesional skin. ***(B1)*** In addition, the change of microenvironment in AE, specifically the increase in pH and flux of oxygen throughout the skin, can impact *S. aureus,* and AE strains should be best suited to take advantage of these microenvironment changes. Because *S. aureus* can be present within healthy skin, there must be a differential strain response to AE-like environmental changes between healthy and AE strains. ***(C1)*** I hypothesize that the healthy and AE *S. aureus* strain secretions are indistinguishable in healthy environmental conditions (acidic pH and anoxic). ***(D1)*** Lastly, the current non-invasive sampling standard (pre-wet swabs) for skin metabolome collection is not feasible for transition into clinics due to its time-intensive processing. I aim to develop a new sampling method that is comparable to the current non-invasive sampling standard.

Methods: Tape strips were taken from healthy, AE, psoriasis, and Chronic Spontaneous Urticaria participants and the total abundance of bacteria across the skin layers was measured by qPCR. The 16S rRNA gene was used to determine the bacterial load, and two other targets for Staphylococci and *S. aureus* were also measured within the samples. Two studies were used for the measurement of the anaerobic microbiome: the CK-AD study[1], which focused on depth differences in healthy skin, and the ProRaD study[2], which focused on observing changes in AE. From these studies, compositional data, measured by 16S rRNA next-generation sequencing, was cross-referenced to the literature to annotate the oxygen status of each OTU. Afterward, the relative abundance of the anaerobic OTUs at the surface and within the skin and between healthy and AE samples taken at the skin's surface was quantified. For determining the environmental (pH and oxygen) impact on *S. aureus*, participant strains isolated from healthy and AE individuals were grown in low pH aerobic, neutral pH aerobic, neutral pH anoxic, and oxygen fluctuation conditions. The area under the curve was calculated from the growth curves and used for comparison of growth. Furthermore, for 30 (15 healthy, 15 AE) strains, the secretions were collected and measured by UPLC-MS[2]. Finally, WET PREP and pre-wet swab samples were taken from the skin of healthy individuals (n=22) and run on

[1] The CK-AD study is a study that was focused on connecting epidermal barrier dysfunction, as measured through the skin's transcriptome, to microbiome signatures, measured by RNA sequencing, in AE and healthy participants. Only three healthy participants in the study had the deeper skin sampled, and this portion will be used for the research within this thesis. The study has been published, see (Altunbulakli et al. 2018).

[2] The ProRaD study is a multi-center, time-course observational study focused on AE, described in Bieber et al. (Thomas Bieber et al. 2020) and found at https://ck-care.ch/en/studies/pro-rad-study/. Participants in the study will be observed for five years. Data used from this cohort consists of AE - LS (n = 149) and NL (92) - and healthy (HE, 35) samples.

UPLC-MS^2. A follow-up study comparing WET PREP and bare skin volatilome measurement, along with the volatilome of AE and healthy skin, was performed using a SICRIT-MS. All data were analyzed using either MetaboAnalyst, Prism, or R.

Results: From the investigation in this thesis, the data clearly shows that the micro-environment is an essential factor in skin health. From the microbial side, bacteria are detected throughout the layers of the epidermis, with a higher bacterial load found in AE samples. In addition, the absolute abundance of Staphylococci is increased in AE and psoriasis and correlates to severity. *S. aureus* is specifically abundant within AE skin and can be found across the layers of the skin. In addition, the anaerobic microbiome is lost within AE skin, and its loss correlates to AE severity. With regards to *S. aureus* growth, within scratching (oxygen flux) conditions, there is a larger growth of AE *S. aureus* strains, and the location of isolation plays a significant role in how much the environment impacts bacterial growth. In addition, the metabolite secretions from *S. aureus* differ according to the host's skin status. Healthy and AE strain differences are best seen for the shift in the pH than for changes in oxygen levels. Regarding skin metabolomics, the sampling method does impact the capture of the skin's metabolites. Besides lipid detection, WET PREP is comparable to pre-wet swabs and can measure the skin volatilome and distinguish AE and healthy skin.

Conclusion: Regarding microbiome studies, the skin should not be considered only at its surface but as a whole because differentiation in bacterial load is seen across its layers. In addition, because the bacterial load is increased in skin diseases with a known barrier disruption, further research should look deeper into the composition of the data to determine if this rise of bacteria is due to select species or only a group of bacteria. The anaerobic microbiome appears to have been lost in AE, and whether this loss is inherent to AE skin or simply a result of scratching should be further explored. Since no select anaerobic species contributes to the differences between healthy and AE skin, the impact of one select species should not be the focus of therapies. Instead, the focus should be restoring the biome as a whole. Regulating oxygen and pH levels in the skin is critical to preventing *S. aureus* overgrowth; these environments can distinguish between healthy and AE strains. Other therapies on AE should consider a joint environmental approach, and the metabolites highlighted here should be further explored to confirm clinical relevance. In addition, WET PREP has shown great promise in its use for diagnosing AE, and follow-up studies are underway to create this diagnostic.

Zusammenfassung

Hintergrund: Das atopische Ekzem (AE) ist eine entzündliche Hauterkrankung, von der weltweit etwa 20% der Kinder betroffen sind und deren frühes Auftreten zur späteren Entwicklung von Asthma und Allergien führt. Es hat sich gezeigt, dass genetische und umweltbedingte Faktoren zur Entwicklung von AE beitragen. Genauer gesagt ist der pH-Wert der Haut bei AE erhöht, Mikrowunden - verursacht durch Kratzen - verändern die Verfügbarkeit von Sauerstoff in der Haut, und es kommt zu einer übermäßigen Vermehrung von *S. aureus* bei AE. Derzeit konzentriert sich ein großer Teil der Forschung darauf, wie sich das Mikrobiom bei AE verändert und wie *S. aureus* das Immunsystem bei AE beeinflusst. Obwohl die Haut ein vielschichtiges Organ ist, konzentrieren sich Mikrobiom-Studien häufig auf die Hautoberfläche und berücksichtigen nicht die absolute Bakterienmenge, sondern nur die Veränderungen in der Zusammensetzung. Außerdem stimulieren viele immunologische Studien die Haut mit *S. aureus*, entweder epidermal oder dermal, ohne die tatsächliche Lokalisierung von *S. aureus* in der erkrankten Haut zu kennen. Die Bakterien befinden sich in den tieferen Schichten der Haut, weil in der Haut ein Sauerstoffgefälle existiert, das das Wachstum anaerober Bakterien begünstigen kann. Die Mikroverletzungen bei AE würden in der Folge zu einem Verlust dieses Sauerstoffgradienten führen. Das anaerobe Mikrobiom ist in läsionaler Haut bei AE reduziert. Allerdings ist derzeit nicht bekannt, ob diese Verringerung spezifisch für die läsionale Haut ist und in welchem Zusammenhang sie mit dem Schweregrad der AE steht. Darüber hinaus gibt es nicht nur einen bestimmten Typ von *S. aureus*, sondern mehrere Stämme. Während eines AE-Schubs kolonisiert ein klonaler Typ die Haut. Außerdem kann ein Teil der gesunden Bevölkerung *S. aureus* in sich tragen, ohne AE zu entwickeln, was auf eine Differenzierung zwischen gesunden und atopischen Stämmen hindeutet. Darüber hinaus gibt es zwar verschiedene Methoden zur Messung des Hautmetaboloms, doch sind diese unterschiedlich invasiv, wobei die derzeitige nicht-invasive Methode, der vorgängig angefeuchtete Abstrich, eine einwöchige Vorbereitung vor der Probenahme erfordert, was eine Übertragung in den klinischen Bereich unmöglich macht.

Hypothese und Ziele: Ziel dieser Arbeit ist es, die Auswirkungen der Umwelt (Mikrobiom, Sauerstoff, pH-Wert und *S. aureus*) auf die Homöostase der Haut zu untersuchen und eine Methode zur Diagnose der Hautgesundheit zu entwickeln. ***(A1)*** Ich stelle die Hypothese auf, dass die bakterielle Belastung in den verschiedenen Hautschichten und innerhalb der Hautkrankheiten unterschiedlich ist. ***(A2)*** Da die Zusammensetzung von *S. aureus* in der Regel bei AE übermäßig ist, gehe ich davon aus, dass dieser in AE in größerer absoluter Menge

vorhanden ist. Da die Bakterien in allen Hautschichten zu finden sind und es ein unterschiedliches Sauerstoffgefälle in der Haut gibt, ***(A3)*** sollte es außerdem ein anaerobes Mikrobiom in der Haut geben. Die AE-Haut weist Mikroverletzungen und eine Abnahme von Strukturproteinen wie Tight Junctions und Filaggrin auf. ***(A4)*** Dies würde hypothetisch zu einem Verlust des Sauerstoffgradienten in der Haut und folglich zu einem Verlust des anaeroben Mikrobioms sowohl in der läsionalen als auch in der nicht-lesionalen Haut führen. Darüber hinaus kann sich die Veränderung der Mikroumgebung bei AE, insbesondere der Anstieg des pH-Werts und des Sauerstoffflusses durch die Haut, auf *S. aureus* auswirken. Es wird angenommen, dass AE-Stämme am besten geeignet sind, diese Veränderungen der Mikroumgebung zu nutzen. ***(B1)*** Da *S. aureus* auch in gesunder Haut vorkommen kann, muss es eine unterschiedliche Reaktion des Stammes auf AE-ähnliche Umweltveränderungen zwischen gesunden und AE-Stämmen geben. ***(C1)*** Ich stelle die Hypothese auf, dass die Sekrete des gesunden und des AE-Stamms von *S. aureus* unter gesunden Umweltbedingungen (niedriger pH-Wert und anoxisch) nicht zu unterscheiden sind. ***(D1)*** Schließlich ist der derzeitige nicht-invasive Probenahme-Standard (vorgenässte Abstriche) für die Hautmetabolom-Sammlung aufgrund der zeitaufwändigen Verarbeitung nicht Anwendung in Kliniken geeignet. Mein Ziel ist es, eine neue Probenahmemethode zu entwickeln, die mit dem derzeitigen nicht-invasiven Probenahmestandard vergleichbar ist.

Methoden: Es wurden Klebebandstreifen von gesunden (HE), AE-, Psoriasis- und CSU-Teilnehmern entnommen, und die Gesamthäufigkeit von Bakterien in den verschiedenen Hautschichten wurde mittels qPCR gemessen. Das 16S rRNA-Gen wurde zur Bestimmung der bakteriellen Belastung verwendet, und zwei weitere Ziele für Staphylokokken und *S. aureus* wurden ebenfalls in den Proben gemessen. Zur Messung des anaeroben Mikrobioms wurden zwei Studien herangezogen: die CK-AD-Studie[3], die sich auf Tiefenunterschiede in gesunder Haut konzentrierte, und die ProRaD-Studie[4], die sich auf die Beobachtung von Veränderungen bei AE konzentrierte. Aus diesen Studien wurden die mittels 16S rRNA-Sequenzierung der

[3] Die CK-AD-Studie ist eine Studie, die sich darauf konzentrierte, eine Verbindung zwischen der epidermalen Barrieredysfunktion, die durch das Transkriptom der Haut gemessen wurde, und den Mikrobiomsignaturen, die durch RNA-Sequenzierung gemessen wurden, bei AE und gesunden Teilnehmern herzustellen. Nur bei drei gesunden Studienteilnehmern wurde die tiefere Haut beprobt, und dieser Teil wird für die Forschung in dieser Arbeit verwendet. Die Studie ist veröffentlicht worden, siehe (Altunbulakli et al. 2018).

[4] Bei der ProRaD-Studie handelt es sich um eine multizentrische Beobachtungsstudie mit Schwerpunkt auf AE, die in Bieber et al. (Thomas Bieber et al. 2020) beschrieben und unter https://ck-care.ch/en/studies/pro-rad-study/ zu finden ist. Die Teilnehmer an der Studie werden über einen Zeitraum von fünf Jahren beobachtet. Die verwendeten Daten aus dieser Kohorte bestehen aus AE - LS (n = 149) und NL (92) - und gesunden (HE, 35) Proben.

nächsten Generation gemessenen Zusammensetzungsdaten mit der Literatur abgeglichen, um den Sauerstoffstatus der einzelnen OTUs zu annotieren. Anschließend wurde die relative Häufigkeit der anaeroben OTUs an der Hautoberfläche und innerhalb der Haut sowie zwischen gesunden und AE Proben an der Hautoberfläche quantifiziert. Um den Einfluss der Umwelt (pH-Wert und Sauerstoff) auf *S. aureus* zu bestimmen, wurden die aus gesunden und AE Personen isolierten Teilnehmerstämme unter aeroben Bedingungen mit niedrigem pH-Wert, neutralem pH-Wert, anoxischem pH-Wert und Sauerstoffffluktuation gezüchtet. Der Wachstumserfolg wurde anhand der Fläche unterhalb der Kurve gemessen, die aus den Wachstumskurven berechnet wurde. Außerdem wurden für 30 Stämme (15 HE, 15 AE) die Sekrete gesammelt und mittels UPLC-MS^2 gemessen. Schließlich wurden von der Haut gesunder Personen (n=22) WET-PREP- und Vor-Nass-Abstrichproben entnommen und mit UPLC-MS^2 untersucht. Eine Folgestudie, in der die Volatilom-Messungen von WET PREP und nackter Haut sowie die Volatilom-Messungen von AE und gesunder Haut verglichen wurden, wurde mit einem SICRIT-MS durchgeführt. Alle Daten wurden entweder mit MetaboAnalyst, Prism oder R ausgewertet.

Ergebnisse: Aus der Untersuchung in dieser Arbeit geht eindeutig hervor, dass die Umwelt ein wesentlicher Faktor für die Hautgesundheit ist. Was die mikrobielle Seite betrifft, so werden Bakterien in allen Hautschichten nachgewiesen, wobei eine höhere bakterielle Belastung in AE- und Psoriasis-Proben festgestellt wurde. Darüber hinaus ist die absolute Häufigkeit von Staphylokokken bei AE und Psoriasis erhöht und korreliert mit dem Schweregrad. Insbesondere *S. aureus* scheint in der AE-Haut besonders häufig zu sein und ist in allen Hautschichten auffindbar. Darüber hinaus geht das anaerobe Mikrobiom in der AE-Haut verloren, und sein Verlust korreliert mit dem Schweregrad der AE. Was das Wachstum von *S. aureus* betrifft, so ist unter den Bedingungen des Kratzens (Sauerstoffzufuhr) ein größeres Wachstum von *S. aureus*-Stämmen bei AE zu beobachten. Der Ort der Isolierung spielt eine wichtige Rolle dabei, wie stark die Umgebung das Bakterienwachstum beeinflusst. Darüber hinaus unterscheiden sich die Metabolitensekrete von *S. aureus* je nach Hautstatus des Wirts, und die Unterschiede zwischen Gesundheits- und AE-Stämmen lassen sich am besten bei der Verschiebung des pH-Werts als bei Veränderungen des Sauerstoffgehalts erkennen. Was die Hautmetabolomik betrifft, so wirkt sich die Probenahmemethode auf die Erfassung der Hautmetaboliten aus. Neben dem Lipidnachweis ist WET PREP vergleichbar mit vorgenässten Abstrichen und kann das Hautvolatilom messen und AE von gesunder Haut unterscheiden.

Schlussfolgerung: Bei Studien zum Mikrobiom sollte die Haut nicht nur an der Oberfläche, sondern als Ganzes betrachtet werden, da sich die Bakterienbelastung in den verschiedenen Hautschichten unterscheidet. Da die bakterielle Belastung bei Hautkrankheiten mit einer bekannten Barrierestörung erhöht ist, sollten weitere Untersuchungen die Zusammensetzung der Daten genauer untersuchen, um festzustellen, ob dieser Anstieg der Bakterien auf ausgewählte Arten oder nur auf eine Gruppe von Bakterien zurückzuführen ist. Das anaerobe Mikrobiom scheint bei AE verloren gegangen zu sein, und es sollte weiter untersucht werden, ob dieser Verlust mit der AE-Haut zusammenhängt oder einfach eine Folge des Kratzens ist. Da keine ausgewählte anaerobe Spezies zu den Unterschieden zwischen gesunder und anaerober Haut beiträgt, sollten die Auswirkungen einer ausgewählten Spezies nicht im Mittelpunkt der Therapien stehen. Stattdessen sollte der Schwerpunkt auf der Wiederherstellung des Bioms als Ganzes liegen. Die Regulierung der Sauerstoff- und pH-Werte in der Haut ist für die Verhinderung eines übermäßigen Wachstums von *S. aureus* von entscheidender Bedeutung; diese Umgebungen können zwischen gesunden und aeroben Stämmen unterscheiden. Andere Therapien zur Behandlung von AE sollten einen gemeinsamen Umweltansatz berücksichtigen, sowie eine weitere Untersuchung der hier hervorgehobenen Metaboliten könnte erfolgen, um ihre klinische Relevanz zu bestätigen. Darüber hinaus hat sich WET PREP als sehr vielversprechend für die Diagnose von AE erwiesen, und es laufen derzeit Folgestudien zur Entwicklung dieser Diagnose.

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List of Abbreviations

Abbreviation	Term
AE	Atopic Eczema
AMP	Antimicrobial peptides
ASCA	ANOVA simultaneous component analysis
AUC	Area under the Curve
CFU	Colony forming units
CoN	Coagulase negative
CSU	Chronic Spontaneous Urticaria
DAHSS	Differences in Atopic and Healthy Strain Secretions
DC	Dendritic Cells
EASI	Eczema Area and Severity Index
FLG	Filaggrin
HBD-2	Human β-defensin 2
HE	Healthy
HIF-1α	Hypoxia-inducible factor 1α
HILIC	Hydrophilic interaction chromatography
Hrs	Hours
IEM	Institute of Environmental Medicine
IL	Interleukins
IRF	Interferon regulatory factor

ISF	Interstitial fluid
LB	Luria Broth
LC	Langerhans cells
LC-MS^2	Liquid chromatography – mass spectrometry
LS	Lesional
Min	Minutes
MLST	Multi-locus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Microsoft
MSA	Mannitol Salt Agar
N.A.	Not applicable
n.d.	No data
NEG1	Technical negative controls
NEG2	Biological negative controls
NK	Natural killer T cells
NL	Non-lesional
ns	Non-significant
NS	Netherton syndrome
OTU	Operational taxonomic unit
P	Psoriasis
PASI	Psoriasis Area and Severity Index
PASTURE	Protection Against Allergy Study in Rural Environments
PCA	Principal Component Analysis
ProRaD	Prospective longitudinal study to investigate the Remission phase in patients with Atopic Dermatitis
PRR	Pattern recognition receptors
QC	Quality Control
qPCR	Quantitative PCR
qPCR	Quantitative Polymerase Chain Reaction
SCFAs	Short Chain Fatty Acids
SCORAD	Scoring Atopic Dermatitis
Sec	Seconds
$s\Delta O_2$	pH 7.0, aerobic versus pH 7.0, anaerobic
sΔpH	pH 5.5, aerobic versus pH 7.0, aerobic
TEWL	Transepidermal water loss
Th	T helper
TLR	Toll-like receptors
Tm	Memory T cells
Treg	T regulatory
Trm	Resident Memory T cells
TS2	2^{nd} tape strip
TS20	20^{th} tape strip
TS40	40^{th} tape strip
TS60	60^{th} tape strip

TS80	80^{th} tape strip
TSASA	Time series analysis of *Staphylococcus Aureus*
TSLP	Thymic Stromal Lymphopoietin
UPLC-MS^2	Ultra-performance liquid chromatography coupled to mass spectrometry
VOCs	Volatile organic compounds

Chapter 1 - General Introduction

As the skin is easily viewed without the need for specialized equipment or techniques, the history of research and treatment of the skin extends as far back as 1550 B.C. with the Ebers Papyrus discovered at Thebes (Ferreira, Weber, and Bonamigo 2021). Hippocrates, often referred to as the father of medicine, was the first to classify skin diseases based on their origin, using the terms idiopathic, referring to diseases with a visible origin on the skin, and exanthematic, referring to those diseases which seem to bloom outwards from inside the body (Ferreira, Weber, and Bonamigo 2021; Ladda and Lynde 2019). From the 18th to 19th century, several advancements in dermatology were made. Of note are three publications, *Doctrina de Morbis Cutaneis* by Joseph Jacob Ritter von Plenck in 1776, *Tractatus de Morbis Cutaneis* by Anne Charles Lorry in 1777, and the *Atlas der Hautkrankeiten* by Ferdinand von Hebra in 1856 (Azulay 2003; Ferreira, Weber, and Bonamigo 2021). *Doctrina de Morbis Cutaneis* was the first published book on Dermatology and covered 150 skin diseases (Azulay 2003; Ferreira, Weber, and Bonamigo 2021). *Tractatus de Morbis Cutaneis* focused on internal and external pathologies of the diseases, rejecting the former theory of humors (black bile, yellow bile, phlegm, and blood) and covered the skin's anatomy alongside disease pathology (Azulay 2003; Everett 1979). In addition, Lorry was the first to consider the skin as an organ and generated theories on the influence of the macro-environment on the skin (Everett 1979). Hebra was a famous dermatologist in Vienna who mentored many pioneers in the field, and the *Atlas der Hautkrankeiten* was the first German atlas of dermatology (Ferreira, Weber, and Bonamigo 2021). At the beginning of the 20th century, the role of fungi in causing skin disease was becoming more solidified, and novel treatments such as ultra-violet light therapy came into popularity (McCaw 1944). In addition, in the 20th and 21st centuries, discussions of the role of bacteria in skin health earnestly began and continue to be subjects of discussion (Swaney and Kalan 2021). Since 1550 B.C. there have been many innovations within the field of dermatology, and there are still many mysteries as to the pathogenesis of many skin diseases. This thesis will cover the aspects of the micro-environment on the health of the skin and its homeostasis.

1.1 Factors of Skin Homeostasis

1.1.1 Physical

As our barrier to the outside world, the skin is the largest organ in the human body and has several factors contributing to its health. Physically the skin is divided into three main parts:

the epidermis (the outermost layer), the dermis, and the hypodermis. The epidermis comprises five layers, from the outside to the inside: the stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale, visualized in Figure 1.1. The skin divides itself from the stratum basale and cornifies as it is pushed upwards to the stratum corneum. The segments of the skin are not evenly divided in size (R. Wong et al. 2016), and for the smallest layer, the epidermis, its thickness is influenced by body location, sex (disputed), age, and individual variation (Sandby-Møller, Poulsen, and Wulf 2003; Lintzeri et al. 2022). In addition, hair follicles and sweat glands in the skin span from the epidermis to the dermis, and sebaceous glands lie within the dermis. All of this makes up the first divisions of the skin.

Because the skin forms a cellular barrier from the environment, several factors go into maintaining the physical connectivity of the skin. To form that cutaneous barrier, keratinocytes use (1) tight junction complexes (Simpson, Patel, and Green 2011; Katsarou et al. 2023; Nguyen and Soulika 2019), (2) filaggrin (FLG) (Simpson, Patel, and Green 2011; Furue 2020), and (3) the cornified envelop (Simpson, Patel, and Green 2011). Many factors go into each part of the cutaneous barrier, and here I will briefly review the broader concepts. Tight junctions are transmembrane proteins connecting keratinocytes among the stratum granulosum (Katsarou et al. 2023). Tight junctions also regulate molecular exchange between keratinocytes and control the skin's permeability (Katsarou et al. 2023). FLG is a structural protein that resides among the stratum corneum, and its uncleaved precursor protein, pro-filaggrin, resides among the stratum granulosum (Simpson, Patel, and Green 2011; Furue 2020). FLG has a role in maintaining skin hydration (T. Matsui et al. 2011; Sandilands et al. 2009), pH (Proksch 2018a) and is the scaffold for the cornified envelope (Simpson, Patel, and Green 2011). The cornified envelope is comprised of Keratins 1 and 10, desmosomes, loricrin, and involucrin (Furue 2020). It is a hydrophobic barrier for the skin and has redundancies in loricrin and involucrin that prevent one mutation from destroying the entire layer (Simpson, Patel, and Green 2011; Furue 2020).

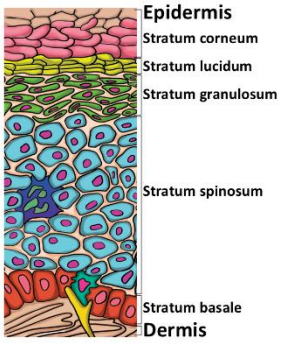


Figure 1.1 Divisions of the Skin

Figure modified from (Yousef, Alhajj, and Sharma 2023). The skin is divided into three major parts: the epidermis, dermis, and hypodermis. Visualized here are the divisions within the epidermis: the stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale in descending order from the outer surface of the skin to within.

1.1.2 Chemical

Alongside the physical barrier, there is also the chemical barrier of the skin consisting of natural moisturizing factors, salt, pH, and oxygen. The skin's pH gradient rises from the acidic skin surface to the blood (Proksch 2018a). More specifically, within the stratum corneum, the pH increases around two units from the surface to deeper layers (Schreml et al. 2011; Turner, Cullander, and Guy 1998). In a more detailed study, this gradient was found to be parabolic, where the lowest pH can be found within the stratum corneum (personal communication about unpublished data from Seo-yeon Moon, et al. and (Wohlrab, Gebert, and Neubert 2018)). At the skin's surface, the pH ranges from 4 to 6 (Finnegan, Duffy, and Morrin 2022; Schmid-Wendtner and Korting 2006; Proksch 2018a; Ali and Yosipovitch 2013). This low pH can inhibit the colonization of pathogenic organisms (Skowron et al. 2021; Proksch 2018b) and aids in controlling skin differentiation (Proksch 2018b; Ali and Yosipovitch 2013). pH is also influential in maintaining the lipid barrier in the skin, where sphingomyelinase and beta-glucocerebrosidase - involved in ceramide synthesis - are pH-dependent (Ali and Yosipovitch 2013). In addition, based on in vivo studies, a lower pH helps with the adhesion of resident skin flora to the skin (Lambers et al. 2006).

Several factors control and impact the skin's pH. As hinted earlier, the filaggrin-histidine-urocanic acid pathway can control the skin's pH with FLG breakdown, acidifying the skin via amino acids (Afghani et al. 2022; Proksch 2018a). In addition, the breakdown of phospholipids, by secretory phospholipase 2, into free fatty acids can acidify the stratum corneum (Fluhr et al. 2004; Ilic et al. 2014). Lastly, the Na^+ /H^+ antiporter (NHE1) can acidify the skin, and loss of this pump in older skin coincides with a pH increase (Choi et al. 2007;

Fluhr et al. 2004). Washing the skin with soap can increase the skin's pH (Lambers et al. 2006), and salicylic acid and tape stripping decrease the skin's pH (Finnegan, Duffy, and Morrin 2022). However, the skin's buffering capacity, over time, is relatively high (Hülpüsch et al. 2020a; Turner, Cullander, and Guy 1998), whereas only products with high buffering capacity can effectively modulate skin pH (Proksch 2018a; Brinke et al. 2021). Despite that, it is much easier to alkalize the skin than to acidify it (Zhai et al. 2009), possibly due to the mechanisms behind the skin's buffering. CO_2, keratin, sebum, and amino acids have all been suggested as buffering agents within the skin (Levin and Maibach 2008). The latter, amino acids, is the only agent not disputed in its contribution (Levin and Maibach 2008). Amino acids are produced from the breakdown of FLG and other proteins within the skin (Levin and Maibach 2008; Levin, Friedlander, and Del Rosso 2013a). Overall, within healthy skin, there is a stable system maintaining its acidic mantle.

Alongside the acidic mantle is a hypoxic mantle within the skin. Although the skin is exposed to atmospheric oxygen, the lack of oxygen is crucial for skin function. In vitro keratinocyte studies show that anoxic conditions, i.e., no oxygen, can prolong the cells' lifespan, and cells are still viable in this environment (Kino-oka et al. 2005). Under hypoxic conditions, i.e., low oxygen, in vitro keratinocytes better reflected the typical activity observed in the stratum basale (Ngo et al. 2007). In addition, when grown in hypoxic conditions, human skin equivalents better mimic native human skin, according to their ceramide composition and epidermal differentiation (Mieremet et al. 2019). Finally, the lack of oxygen can even assist in maintaining a healthy skin state. Mild hypoxia prevents the proliferation of mutagenic, i.e., cancerous cells, by inducing cell apoptosis of those cells in primary normal human keratinocytes (Nys et al. 2012; 2011). The location of the anoxic layer appears to lie within the epidermis. A study by Evans et al. found that the epidermis and hair follicles are hypoxic (Evans et al. 2006). Hypoxia-inducible factor 1α (HIF-1α) is a transcription factor that is deactivated according to oxygen presence because it degrades when oxygen is present. NO and H_2O_2 can prevent oxygen-based degradation, but overall, HIF-1α appears to be a good metric for locating the anoxic environment in the skin (Karlenius and Tonissen 2010). HIF-1α has been discovered within the basal layer of the epidermis (Rezvani et al. 2011) and can induce FLG gene expression (W. J. Wong et al. 2015), indicating that low oxygen levels can regulate the pH skin barrier. These low oxygen conditions can also assist in the growth of skin commensals which is covered in more detail within section 1.1.5. Overall, this shows the presence and benefits of the hypoxic mantle.

1.1.3 Immune

The skin has a resident immune system and multiple different types of skin-resident immune cells. The immune response is divided into innate (non-specific) immunity and adaptive (specific) immunity, which have components within the epidermis and dermis. Keratinocytes and Langerhans cells (LC) are a part of the innate immune response. Keratinocytes can recognize invaders by their pathogen-associated molecular patterns (Nestle et al. 2009). This recognition occurs at their pattern recognition receptors (PRR), such as Toll-like receptors (TLR) (Gallo and Nakatsuji 2011), which are transmembrane proteins on the outside of the cell (Daëron 2022). After recognition of pathogens, keratinocytes then produce antimicrobial peptides (AMP) and secrete cytokines like interleukins (IL) and thymic stromal lymphopoietin (TSLP) to signal for recruitment of cells such as dendritic, T helper (Th), and B cells (Nestle et al. 2009). LC are skin-specific dendritic cells (DC). LC can recognize microbes and, upon activation, create tight junctions with keratinocytes to penetrate through previously established tight junctions in the skin to reach the stratum corneum (Simpson, Patel, and Green 2011). Afterward, LC migrates and primes T cells through antigen presentation (Merad, Ginhoux, and Collin 2008). Residing alongside LC in the stratum spinosum and basale are $CD8^+$ T cells (Simpson, Patel, and Green 2011; Nguyen and Soulika 2019). T cells are a part of the adaptive immune response, and $CD8^+$ T cells recognize antigens presented by keratinocytes and LC (Nestle et al. 2009; Black et al. 2007). In general, after antigen recognition, T cells will both signal using cytokines to recruit more immune cells and kill microbial, infected, or defective cells (Ho and Kupper 2019). There are different types of T cells, such as memory (Tm; including resident memory cells in the skin, Trm), Th, T regulatory (Treg), and natural killer T (NK) cells. Each type has a specialized function. Tm cells remember previous antigens and respond to the enemy cells after activation. Th cells secrete cytokines, for example, IL, to further signal for inflammation (P. Hu et al. 2021), and there are different types of Th cells (Type 1 and Type 2) which secrete unique cytokine profiles to activate different sets of immune cells. Treg cells are a type of Th cells that can turn off the immune response (Kondělková et al. 2010; P. Hu et al. 2021). NK cells are lymphocytes with a T cell receptor and can secrete cytokines TNF and IFNγ and induce apoptosis of enemy cells (Nestle et al. 2009). LC, keratinocytes, and $CD8^+$ T cells are the immune cells within the epidermis.

Regarding the dermis, there are DC, macrophages, mast cells, and NK cells. DCs are a part of the innate immune response, and like the epidermal DC, DC are antigen presenters and induce the proliferation of T cells. Unlike epidermal DC, dermal DC interact with follicular Th

cells and B cells (Merad, Ginhoux, and Collin 2008). Another member of the innate immune response is macrophages which are scavengers that recognize, phagocyte, and degrade bacteria, defective, and dead cells and can also activate T cells (Hirayama, Iida, and Nakase 2017; Yanez et al. 2017). B cells are a part of the adaptive immune response. Secretion of immunoglobulin E (IgE) antibodies by B cells can activate mast cells to degranulate, secrete cytokines, and directly interact with keratinocytes (Voss et al. 2021). In bacterial infections, mast cells can recognize TLR and release AMP (Voss et al. 2021). Taken together, various cells are involved in the immune barrier of the skin, with differentiation in their localization within healthy skin.

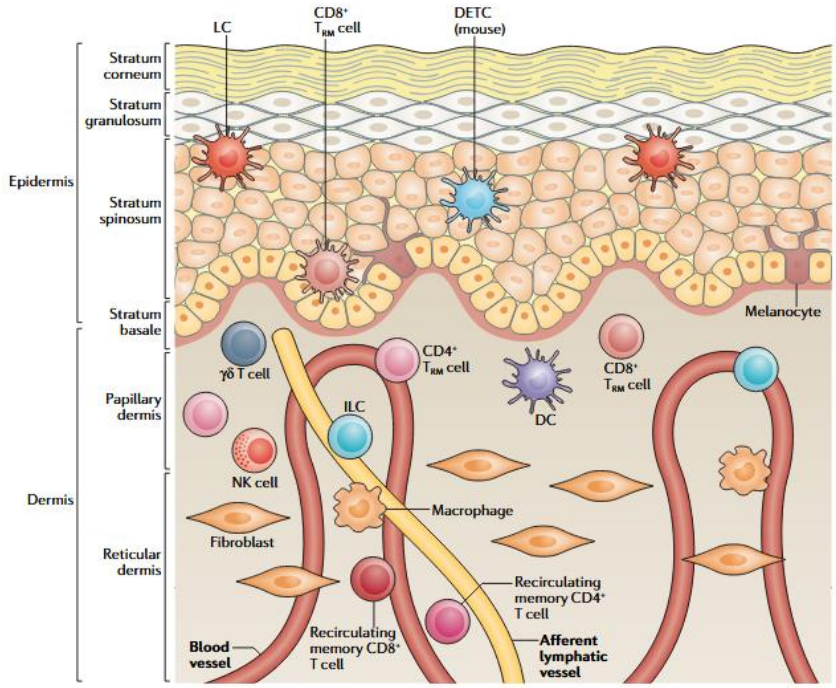


Figure 1.2 Skin Immune System

Figure taken from (Ho and Kupper 2019). The localization of the cells that form the immune system within the skin differ across the skin. Abbreviations: langerhans cells (LC), dendritic cells (DC), T helper (Th), T resident memory (Trm), natural killer T (NK).

1.1.4 Neurological

Along with the immunological system within the skin, there is a neurological barrier, and here we will briefly cover the fundamentals of this skin barrier. The neurological barrier is connected to the peripheral, autonomous, and central nervous system (Roosterman et al. 2006). Within the skin lies sensory nerves, and they are responsible for recognizing the sensations of temperature, pressure, and itch, to name a few (Ansel et al. 1997; Roosterman et al. 2006). These nerves are found in the epidermis and dermis, extending into hair follicles and skin glands (Roosterman et al. 2006). The neurological system within the skin is not just responsible for sending external environmental signals but can also interact with the immune system

(Steinhoff et al. 2003; Ansel et al. 1997; Paus, Theoharides, and Arck 2006). When sensory nerves are activated, they send a pulse to the central nervous system, and afterward, another signal is sent in the reverse direction where, for example, neuropeptides are released (Ansel et al. 1997). These neuro-mediators, i.e., neuropeptides, neurohormones, and neurotransmitters, can bind to cell receptors on keratinocytes, mast cells, T cells, and B cells and activate cytokine production and antibody secretion (Ansel et al. 1997; Steinhoff et al. 2003). In addition, the communication between the neurological and immune systems is bidirectional. For example, histamine can upregulate the gene expression of a stress-response hormone, corticotropin-releasing hormone (Paus, Theoharides, and Arck 2006). The neurological system within the skin can also sense various environmental changes within the skin. Microbial agents and protons (pH changes) can activate skin neurons (Steinhoff et al. 2003). These are the fundamental concepts of the skin's nervous system.

1.1.5 Microbiome

According to (Whipps, Lewis, and Cooke 1988; Berg et al. 2020), the microbiome is the collection of microorganisms - such as bacteria and fungi - present within a physio-chemical distinct matrix (gut, mouth, skin). For this thesis, the term "microbiome" will refer solely to the bacterial component. The microbiome is integral to skin health through its symbiotic relationship with the immune system (Gallo and Nakatsuji 2011). In early life, the skin microbiome primes the immune system through interaction with T cells, preventing later-in-life aberrant inflammation (Scharschmidt et al. 2015; Weckel et al. 2023; Ho and Kupper 2019). *Staphylococcus epidermidis* is a suspected skin commensal that can prevent inflammation after injury of the skin by secretion of lipoteichoic acid, which binds to TLR2 and blocks the Interferon regulatory factor 3 (IRF3) inflammatory pathway (Gallo and Nakatsuji 2011). In addition, commensal bacteria can prevent the colonization by pathogens through the secretion of AMP (Gallo and Nakatsuji 2011; Nakatsuji et al. 2017; Flowers and Grice 2020), and the combination of microbial and host AMP can more stringently prevent the colonization of pathogens (Flowers and Grice 2020). Finally, through fermentation, anaerobic bacteria produce short chain fatty acids (SCFAs), of which the most abundant are acetic acid, butyric acid, and propionic acid (den Besten et al. 2013). SCFAs are known to control inflammation within the gut, but there is little research on the skin. Of what is known, within the skin butyric acid can activate Treg cells by increasing the expression of G-protein-coupled receptor 43 (Schwarz, Bruhs, and Schwarz 2017), which could then suppress immune system inflammation.

Despite the relative uniformity in the components of the skin, there is variety in terms of sub-structures (hair follicle density, epidermal thickness, presence of sweat and sebaceous glands) within the skin. This variety can create unique microenvironments - sebaceous, dry, and moist - within the skin (Dwyer and Scharschmidt 2022). These microenvironments influence the microbiome's composition (Grice et al. 2009; Costello et al. 2009). In one study by Costello et al., the tongue microbiome was transplanted to the forehead and the forearm (Costello et al. 2009). Over time, the microbiome of the sebaceous forehead resembled the pre-transplantation biome, while at the dry forearm site, the microbiome still resembled the tongue microbiome (Costello et al. 2009). This highlights the importance of the microenvironment in shaping the composition of the skin microbiome, where the capability to shape and influence the skin microbiome depends on the sampling location. Other factors affecting the skin microbiome, summarized in Figure 1.3, are interpersonal variation (Grice et al. 2009; Costello et al. 2009), temporal variation (Grice et al. 2009; Costello et al. 2009), age, sex, hygiene, and external environment (Skowron et al. 2021). Environmental factors are less influential on the dermal microbiome (Bay et al. 2020). Interestingly, many studies sample only the external surface of the skin.

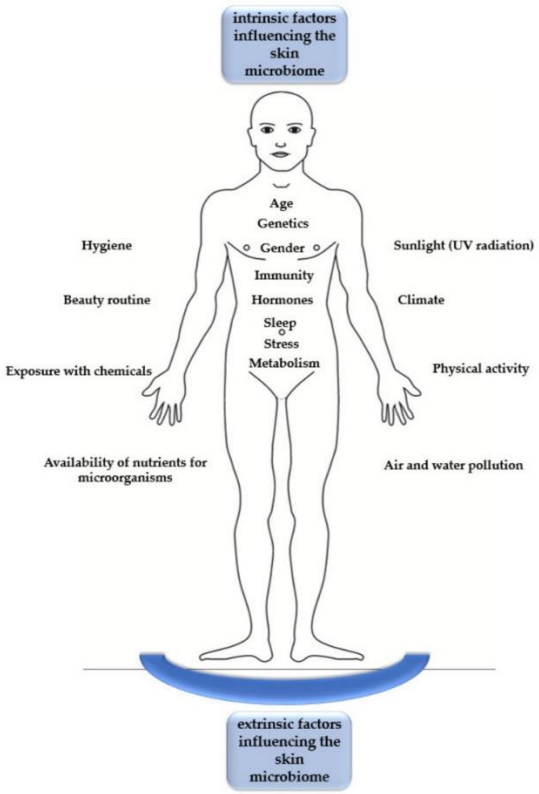


Figure 1.3 Influential Factors of Skin Microbiome

Figure taken from (Skowron et al. 2021). Both intrinsic and extrinsic factors can influence the composition of the skin microbiome. The currently known intrinsic influential factors are age, genetics, gender, immunity, hormones, sleep, stress, and metabolism, while the extrinsic factors are hygiene, cosmetics, chemical exposure, nutrient availability, sunlight (in particular UV radiation), climate, physical activity, and pollution.

The microbiome can penetrate the deeper layers of the skin. The pathogen *S. aureus* can penetrate as far down as the dermis (Nakatsuji et al. 2016). The microbial composition differs in surface versus deeper layers of the stratum corneum (P. L. Zeeuwen et al. 2012). Compared to the dermal microbiome, microbial richness is higher in the epidermis (Bay et al. 2020). A mouse study by Shen et al. suggests that T cells limit the commensal bacteria's colonization of the deeper microbiome. Still, according to the above studies, the microbiome is found in the deeper layers of the skin (Shen et al. 2014). As highlighted above SCFAs can impact the inflammation within the skin, and anaerobic bacteria produce them. The oxygen content within the skin decreases, and based on next-generation sequencing, 16S ribosomal RNA of anaerobes is found within the skin (Bay et al. 2020). Also, anaerobic bacteria can be isolated from the skin (Benediktsdóttir and Hambraeus 1982; Nielsen et al. 1975) and taken together; this suggests a new possible grouping for microorganisms within the skin that impact skin homeostasis. This anaerobic microbiome would be particularly interesting to skin disease because the anaerobic microbiome could elucidate novel pathways involved in disease pathogenesis.

Studies on the microbial load are a valuable supplement to the microbial composition because it helps further the understanding of the microbiome's connection to skin homeostasis. Depending on the location, the skin can harbor 10^3 to 10^6 CFU/cm^2 of bacteria (Skowron et al. 2021). Many studies on the skin microbiome only focus on relative abundances of the taxa, i.e., bacteria, generated by next-generation sequencing. However, relative abundance data should be supplemented with absolute abundance information to validate the previous conclusions (Morton et al. 2019). In addition, skin barrier function, assayed by transepidermal water loss (TEWL), decreases with increasing bacterial load. This suggests that beyond select species contributing to skin health, the bacterial quantity overall can impact skin homeostasis (Jinnestål et al. 2014). Of particular interest are bacteria quantity found across the skin layers and its function in relation to disease, where many studies have focused on the quantification of select taxa but rarely in aggregation to skin depth. Overall, the skin is a complex organ with various paths involved in maintaining homeostasis, resulting in many opportunities for dysfunction and subsequent disease development.

1.2 Atopic Eczema

1.2.1 Brief Introduction of Atopic Eczema

AE is caused by an interplay of genetics and the environment (Nutten 2015). One aspect of the genetic predisposition of AE is due to the downregulation of skin barrier proteins such as FLG, loricrin, and involucrin (Sugiura et al. 2005; Palmer et al. 2006; K. C. Barnes 2010; Agrawal and Woodfolk 2014). FLG mutation is the most dominant relative to the others (Kobayashi and Imanishi 2021). In addition, AE has a Th2 cytokine (Godlewska et al. 2020) and IgE-mediated immune response (Bantz, Zhu, and Zheng 2014; Hill and Spergel 2018). Alongside these host-related drivers of AE, AE arises in conjunction with microbial dysbiosis, where the disturbed microbes can signal inflammation in the affected area (Kobayashi et al. 2015; Park and Lee 2017; Eyerich et al. 2018). Despite these various factors, AE is a complex disease with stratified potential causes for pathogenesis, each described as an AE endotypes, summarized in Table 1.1 (Czarnowicki et al. 2019; Werfel et al. 2016). Because of each endotype's unique differences and overlaps, we can further understand the specific mechanisms involved in each by comparing across these endotypes.

Table 1.1 Endotypes of Atopic Eczema

Table modified from (Afghani et al. 2022). Atopic eczema has been described as having different endotypes, such as IgE mediation, Filaggrin (FLG) level, Staphylococci presence, *S. aureus* influence, and biological race.

Endotype	**Categories**
IgE	Mediated (extrinsic) vs. non-mediated (intrinsic)
FLG	Deficient vs non-deficient
Staphylococci	Present vs. absent
S. aureus	Influenced vs. independent
Biological Race	Asian vs. African vs. European

1.2.2 Impact and Influential Factors

Although AE was first coined in 1933, similar descriptions of the disease can be found as far back as ancient China and ancient Rome (Bhattacharya, Strom, and Lio 2016; Kramer et al. 2017; Taïeb, Wallach, and Tilles 2006). Despite this long history of disease, a cure has not been found, and according to the International Study of Asthma and Allergies in Childhood, a longitudinal epidemiological study covering 56 countries, AE has been increasing in prevalence (H. Williams et al. 1999; Odhiambo et al. 2009; Bantz, Zhu, and Zheng 2014; T. Bieber et al. 2016). AE is an inflammatory cutaneous disease described as itchy, oozing skin

(Roth and Kierland 1964; Bhattacharya, Strom, and Lio 2016). AE is associated with other epithelial disorders like allergy (Gour and Lajoie 2016) and asthma (Roth and Kierland 1964; Bhattacharya, Strom, and Lio 2016; Kramer et al. 2017). AE is described to be the first stage of the atopic march, which is the development of later-in-life atopic disorders due to early life Th2- and IgE-mediated disease (Bantz, Zhu, and Zheng 2014; Hill and Spergel 2018) (Figure 1.4). The outcomes of atopic march are highly related to the age of AE onset, where children who develop AE within the first two years of life have the strongest association with developing asthma and allergies later-in-life. While those who develop AE after two years are more likely to develop allergic rhinitis (Roduit et al. 2017). This is further stratified by the endotypes of AE, where those with the IgE-mediated endotype are more prone to develop asthma and rhinitis than those without (Bantz, Zhu, and Zheng 2014). There are several theories for the pathogenesis of atopic march, summarized in Figure 1.5. 1st, as the skin barrier is weaker in children with AE, this allows for easier penetration of allergens and leads to sensitization of the immune system through TSLP, IL-33, IL-17 and other Th2-related responses (Bantz, Zhu, and Zheng 2014; Hill and Spergel 2018; L. Yang, Fu, and Zhou 2020). 2nd, the lack of exposure to commensal organisms within the skin means that the skin never learned an adequate immune response that is more Th1 skewed (Bantz, Zhu, and Zheng 2014; Gilles et al. 2018). 3rd, genes related to the susceptibility of AE are associated with susceptibility to other allergic diseases (L. Yang, Fu, and Zhou 2020). 4th, because the atopic march connects several epithelial diseases, this disease could be due to an inherent epithelial dysfunction (L. Yang, Fu, and Zhou 2020). The development of AE is also associated with other disorders beyond the atopic march. Those with moderate-to-severe AE have a higher incidence rate of non-melanoma skin cancer, with a 4.1-fold increase in the incidence rate per 1000 people per year for women and a 6.4-fold increase for men (Hedderson et al. 2023). As hinted in Figure 1.4, although AE is described as a pediatric disease with a higher prevalence within children (Silverberg et al. 2021), AE can persist throughout an individual's life. According to Roth and Kierland, after a 20-year follow-up of individuals diagnosed with AE, 60% of individuals with mild AE and 71% with severe disease had continual disease symptoms (Roth and Kierland 1964). Overall, this highlights only a small snippet of the importance of AE and its relevance for study.

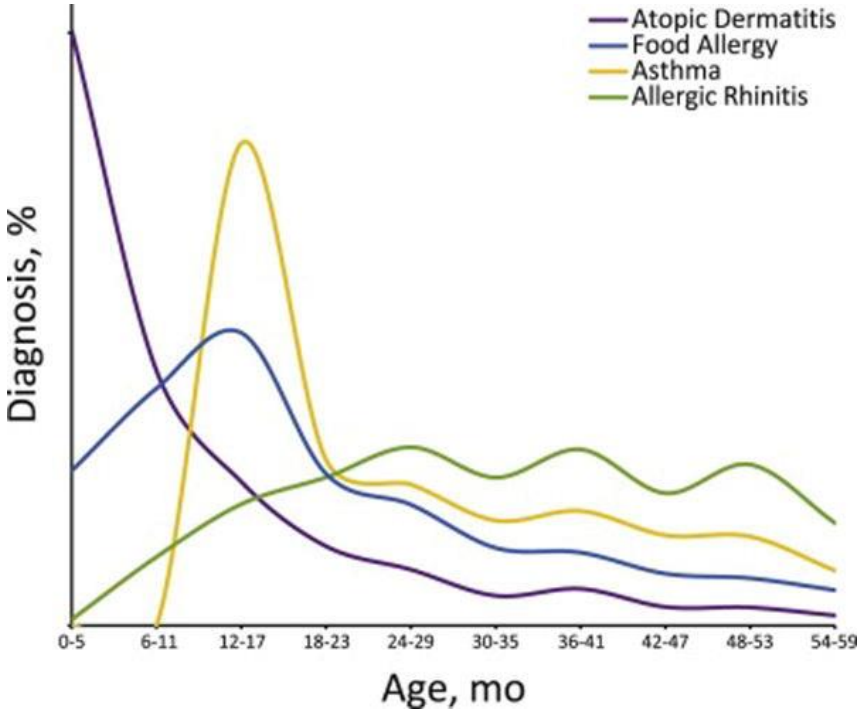


Figure 1.4 Atopic March

Figure taken from (Hill and Spergel 2018). Atopic march has been described as the development of later-in-life atopic disorders due to early life Th2- and IgE-mediated disease. This includes atopic dermatitis, food allergy, asthma, and allergic rhinitis, where as seen in the figure the diagnosis of these four diseases changes as individuals age. In general, there is a trend where the precent that are diagnosed with food allergy, asthma, and allergic rhinitis rises after the diagnosis of atopic dermatitis.

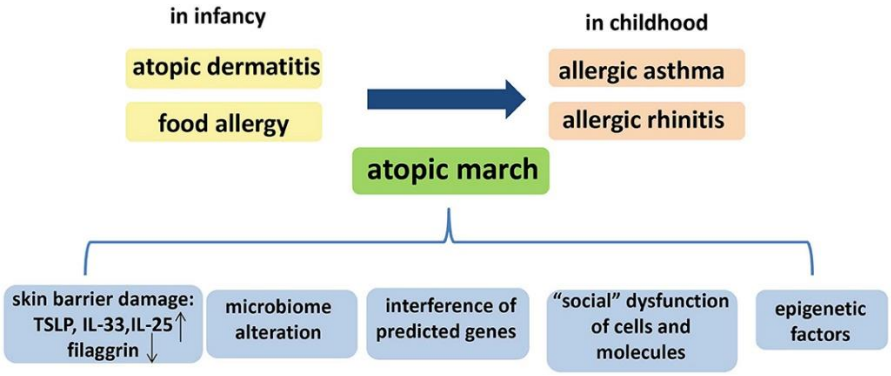


Figure 1.5 Pathways of Atopic March

Figure taken from (L. Yang, Fu, and Zhou 2020). Atopic march has been hypothesized to be due to many different pathways with knowledge still needed on how these pathways intersect. Of those pathways are (1) skin barrier damage due to a rise in thymic stromal lymphopoietin (TSLP), IL-33, IL-25, and a decrease in filaggrin, (2) microbiome alteration whether in the skin or the gut, (3) interference of predicted genes, (4) cellular and molecular dysfunction, and (5) epigenetic factors causing predisposition for these diseases related to atopic march.

There are a variety of factors associated with AE. First, the intrinsic factors will be described, and then the extrinsic. AE is slightly more prevalent in females than males (Roth and Kierland 1964; H. Williams et al. 1999; Odhiambo et al. 2009; Silverberg et al. 2021; Maintz et al. 2021), but this appears to depend on the country of study (Silverberg et al. 2021).

Country of inhabitance has a more significant impact on the prevalence of AE than ethnic grouping (H. Williams et al. 1999), suggesting that environmental exposures may contribute to AE development. This does not dismiss the genetic inheritance of AE, where familial history has long been associated with the disease and is one of the criteria for AE (Roth and Kierland 1964; Bhattacharya, Strom, and Lio 2016; C. Hu et al. 2019). In addition, children with parents who have allergies are more likely to develop AE (Roduit et al. 2017). Downregulation of FLG, the most common mutation in AE, along with involucrin and loricrin, have been found in a portion of AE patients (Sugiura et al. 2005; Palmer et al. 2006; K. C. Barnes 2010; Agrawal and Woodfolk 2014; Kobayashi and Imanishi 2021). Skin barrier genes are not the only mutations in AE. Several susceptibility loci related to IgE have been identified (Cookson and Moffatt 2002), and genetic associations related to Th2 cytokines genes, IL-4 and IL-13, have been discovered (Saito 2005; Dubin, Del Duca, and Guttman-Yassky 2021). Finally, a study of 5297 children from diverse ethnic backgrounds, sub-grouped into Mediterranean, African, and Asian, found that Asian and African descent is positively associated with later-onset and chronic AE and that FLG mutation positively associates with children of Asian ethnicity (C. Hu et al. 2019). There is variation in the FLG locus between African Americans and European Americans, suggesting unique disease mechanisms among these populations (Zhu et al. 2021). Together these hints towards a genetic component to AE, but it appears to be a subgroup within the disease.

AE is also associated with extrinsic factors such as environmental exposures, for example, socioeconomic factors, stress, and urbanization (Luschkova et al. 2021). The lifetime prevalence of AE is higher in those of higher socioeconomic status (Ofenloch et al. 2019). In addition, stress has a cyclical impact on AE suffers. Those afflicted with AE have a lower quality of life, such as sleep disturbances, lifestyle constraints, and continual skin itchiness, which can induce stress, and stress can directly induce AE's immunological profile (Arndt, Smith, and Tausk 2008). This includes increased expression of Th2 cytokines, mast cell-based inflammation, and skin-barrier impairment (Arndt, Smith, and Tausk 2008). In addition, according to an international study based on 18 countries, the prevalence of AE is higher in urban/suburban areas relative to rural (Silverberg et al. 2021). The reason behind this association is multi-factorial. In a study comparing Amish and Hutterites – both being rural communities, but the Hutterites allow for industrial farming – Amish children had decreased asthma prevalence and the presence of protective innate immune signaling (Stein et al. 2016). This suggests that an increase in farm-based exposure can strengthen the innate immune system

and prevent the development of asthma (Stein et al. 2016). Regarding AE, children with farm exposure have reduced incidence of AE, where increased diversity in farm animal and rural environmental exposure had the largest reduction in incidence (Steiman et al. 2020). The birth cohort study, Protection Against Allergy Study in Rural Environments (PASTURE), supports this and found that prenatal exposure to farm animals was also protective against AE (Roduit et al. 2017). Rural exposure is not the only protective measure against AE. Higher bacterial load in drinking water in early life can also protect against the development of AE (Turkalj et al. 2020). These results led to the "hygiene hypothesis," where early exposure to infectious agents can protect against disease, e.g., AE development (Strachan 1989; von Mutius 2010; Hülpüsch et al. 2021). This summarizes a portion of the extrinsic factors associated with AE.

1.2.3 The Role of Microbes in Pathogenesis of Atopic Eczema

Prior exposure to microbes could prevent the colonization of pathogenic organisms associated with AE. Microbe is a general term referring to viruses, fungi, and bacteria. Viral infections, like herpes simplex virus, vaccinia virus, and coxsackie viruses, can occur in AE but only for small populations of AE individuals (P. Y. Ong and Leung 2010; 2016). AE individuals appear to be more susceptible to these viruses, possibly due to cytokine IFN-γ and AMP IL-37 reduction than the viruses driving the disease (P. Y. Ong and Leung 2010; 2016). Another controversial driver of AE is *Malassezia*, which has been found on the skin of AE individuals, and research is ongoing to determine if it induces AE (P. Y. Ong and Leung 2010; Nowicka and Nawrot 2019). Finally, on the bacterial side, *S. aureus* is highly associated with AE (Oh et al. 2013; R. D. Bjerre et al. 2017; Ogonowska et al. 2021) and is overgrown in AE (Cardona, Cho, and Leung 2006). Not all AE individuals carry *S. aureus*; 57–100% of children and 54–100% of adults carry *S. aureus* (Ogonowska et al. 2021). The prevalence of *S. aureus* is more common in the nose relative to the skin of AE individuals (Lehmann et al. 2004). In a study of 66 AE adults, 77.3% had *S. aureus* in both the nose and the skin (Breuer et al. 2002). The re-colonization of *S. aureus* on the skin is suggested to come from the nose (Ogonowska et al. 2021), which is supported by the study of Clausen et al., where the clonal complex of *S. aureus* was identical across the nose, lesional (LS) and non-lesional (NL) skin of AE individuals (Clausen et al. 2017). There are unique factors in colonization that allow AE strains to colonize the skin, further described in section 1.2.4. *S. aureus* colonization can also occur in healthy individuals. In a study of 20 healthy individuals, all had culturable *S. aureus* from the nose but not the skin (Matard et al. 2020). Still, in a larger study by Armstrong-Esther, there were subsets of the healthy population with culturable *S. aureus* from the skin (Armstrong-

Esther 1976); see Figure 1.6. for a breakdown. Despite this healthy colonization, *S. aureus* relative abundance can be predictive of AE disease severity (Hülpüsch et al. 2020b), and counts of *S. aureus* have been associated with severity when measured according to itchiness, patient assessment, whole body Investigator's Global Assessment (Leung et al. 2009), and SCORAD (De Tomassi et al. 2023). One explanation for this could be the stimulation of the immune system by Staphylococci exotoxins containing superantigens, which have been correlated with AE severity (Bantz, Zhu, and Zheng 2014). 50-80% of AE *S. aureus* strains produce superantigens with no one superantigen primarily driving severity (Leung et al. 2009; P. Y. Ong and Leung 2010; 2016). In addition, strains isolated from steroid-resistant AE skin had mutations in exotoxin production (Schlievert et al. 2010). These genes are usually not expressed in anoxic conditions, but the mutations allowed for secretion of exotoxins in anaerobic conditions (Schlievert et al. 2010). In addition to superantigens, *S. aureus* can induce Th2 cytokine IL-33 release (Luo, Lai, and Chang 2023), which is one of the inflammatory markers of AE (Chung et al. 2022). Also, the peptidoglycan that forms the cell wall of *S. aureus* stimulates the infiltration of mast cells into the dermis (K. Matsui and Nishikawa 2005). Microbes have been associated with AE, and *S. aureus* is associated with severity and can stimulate an immune response reflective of AE.

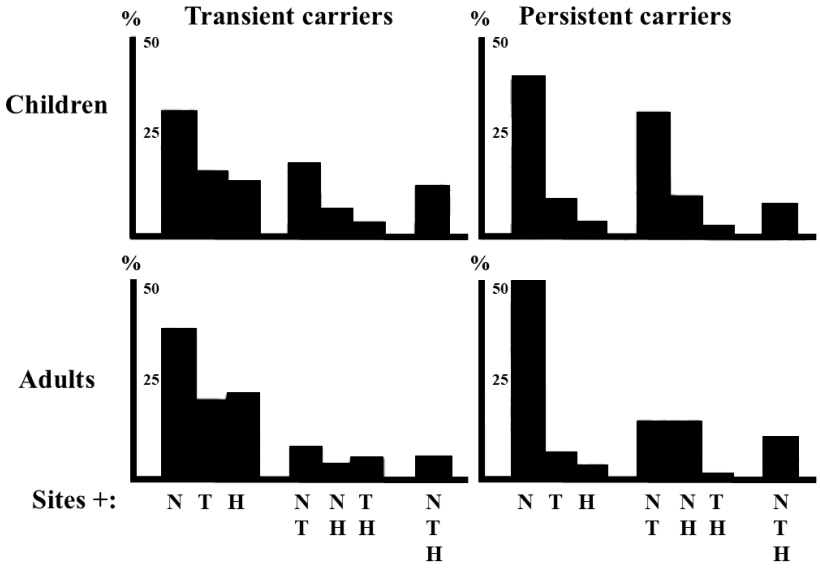


Figure 1.6 Carriage of *S. aureus* from 50 healthy adults and 50 healthy children

Figure modified from (Armstrong-Esther 1976). The distribution of *S. aureus* isolation was determined from the nose (N), throat (T), hands (H), or combinations of these locations according to the percentage of individuals whom has a positive isolation. Both children (top plots) and adults (bottom plots) were included in the study, and according to consistent isolation over time these individuals were separated on whether they were persistent carriers (plots on the right) or transient carriers (plots on the left).

Along with aggravating the immune system, *S. aureus* stimulates the neurological system. Itchiness, also known as pruritus, is a fundamental symptom of AE (Sparavigna, Setaro, and Gualandri 1999; Chovatiya and Silverberg 2022). *S. aureus* abundance has been significantly correlated to itchiness severity, with a Spearman correlation coefficient of 0.306 (Leung et al. 2009). Among topical treatments, the strongest reducer of itchiness was primrose oil (Sher et al. 2012) which has also been found to inhibit *S. aureus* growth (Lodhia, Bhatt, and Thaker 2009). This further supports the connection between itch and *S. aureus*. In addition, when AE individuals are treated with Endobioma^{TM}, a naturally derived protein that selectively targets and kills *S. aureus*, their itchiness decreases by 74% after 14 days (Moreau et al. 2021). *S. aureus* can stimulate itch both indirectly and directly. When keratinocytes come in contact with microbes, they release alarmins (IL-33, TSLP) which can directly stimulate nerve cells to signal itchiness (Legat 2021). In addition, AE *S. aureus* directly induces Glial cells surrounding the neuron because they have pattern recognition receptors that can directly recognize bacteria (Daëron 2022). For *S. aureus* specifically, both α-hemolysin (a secreted pore-forming toxin) and fMIFL (a secreted peptide) could active neurons to signal pain and perhaps similar mechanisms are used to stimulate itchiness (Chiu et al. 2013; Daëron 2022). Despite this connection between *S. aureus* and skin itchiness, *S. aureus* is just one of the various drivers of itchiness in AE, with the others reviewed in Figure 1.7. *S. aureus* appears to have multiple facets that drive AE, but these methods can only occur once *S. aureus* successfully colonizes and overgrows in the skin.

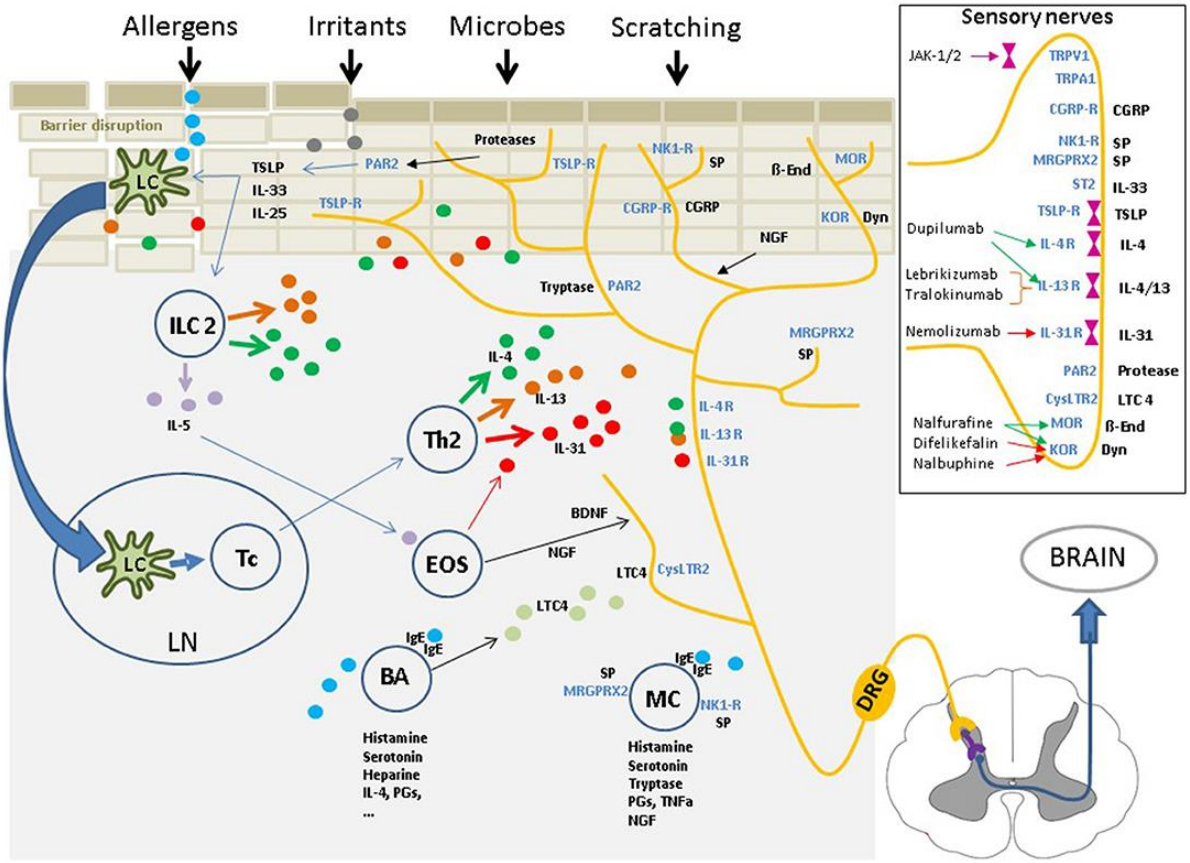


Figure 1.7 Stimulators of Itch and Related Pathways

Figure taken from (Legat 2021). Itch is a common symptom in atopic eczema (AE), and there are several different pathways which have been observed to cause the sensation of itch within this disease. When the skin is exposed to irritants, allergens, microbes, and scratching it causes a cascade of reactions involving the inflammatory response which may result in a downstream cytokine signaling to the neurons' receptors causing the sensation of itch. There are currently several different drugs (JAK1/2, Dupilumab, Lebrikizumab, Tralokinumab, Nemolizumab, Nalfurafine, Difelikefalin, and Nalbuphine) that target cytokine signals and thereby disrupt the sensation of itch in AE.

1.2.4 Potential Causes of S. aureus Colonization

There are several possible reasons for *S. aureus* colonization, summarized in Figure 1.8, one of which is an improper immune response. AE keratinocytes are not able to produce as many AMP relative to psoriasis due to the upregulation of IL-4/IL-13, which can cause AMP suppression, thereby providing an opportunity for pathogens to colonize (P. Y. Ong et al. 2002). AMP human β-defensin 2 (HBD-2) and LL-37 present in psoriasis, another skin disease, can kill AE *S. aureus* strains (P. Y. Ong et al. 2002). The suppression of AMP may be influenced by the host-keratinocyte secretion of alarmins (IL-25, IL-33, and TSLP), which results in IL-13 secretion (Napolitano et al. 2023), or by Staphylococci itself, where staphylococcal enterotoxin B can induce IL-13 expression (Lehmann et al. 2004). In addition, in AE, there is a decrease in IL-17, a known inducer of AMP production in keratinocytes, which could be

another pathway explaining the reduction of AMP in AE (P. Y. Ong and Leung 2016). Improper immune response due to AMP reduction is one of the possible causes of *S. aureus* colonization in AE.

Alongside improper immune response, another cause for *S. aureus* colonization in AE is the loss of the commensal skin microbiome. AE has been described as having microbial dysbiosis regarding evenness, i.e., how evenly the bacterial abundances are distributed (Rauer et al. 2023), and this means that AE has a disbalance in the microbiome. Heavy use of soap and exposure to pollution both result in AE formation, and this is because both collapse the microbial skin barrier (Ahn 2014; Werfel et al. 2016; Tabata, Tagami, and Kligman 1998; T.-Y. Wong 2017; Flohr, Pascoe, and Williams 2005). In addition, AE has been described as a Th2-based inflammatory disease (Saito 2005), and coagulase-negative (CoN) Staphylococci, capable of inhibiting *S. aureus* growth, have reduced colonization in Th2 inflamed skin (Nakatsuji et al. 2023). In *S. aureus* colonized AE skin, there is a reduction in CoN Staphylococci that are capable of inhibiting *S. aureus* by secretion of AMP, like phenol-soluble modulin γ and δ, while in healthy skin, there is an abundance of these bacteria (Gallo and Nakatsuji 2011; Nakatsuji et al. 2017). In addition, skin commensal bacteria, *Cutibacterium acnes* and *S. epidermidis* can directly inhibit *S. aureus* colonization through the secretion of SCFAs (Shu et al. 2013; Traisaeng et al. 2019). SCFAs can pass the bacterial cell membrane and kill *S. aureus* by reducing its intracellular pH (Shu et al. 2013). Commensal bacteria can also directly manipulate the immune system to disfavor *S. aureus* colonization. In mouse models, SCFAs (acetate, butyrate, and propionate) can reduce the IL-33 *S. aureus*-induced inflammation in AE (Luo, Lai, and Chang 2023), which could then resolve the inflammation-based dysbiosis described in (Nakatsuji et al. 2023). Lastly, commensal bacteria can prevent *S. aureus* colonization by inhibiting its quorum sensing system and *S. aureus*-based skin damage (M. R. Williams et al. 2019). The quorum sensing system is responsible for biofilm formation, which is the aggregation of organisms into clumps that generate a unique ecosystem and aid in survival. Overall, the collapse of commensals allows *S. aureus* to invade AE skin, and there are multiple ways commensal bacteria prevent *S. aureus* colonization in healthy skin.

Although damage to the skin barrier creates a new niche for pathogenic colonization, some barrier disruptions are more influential than others. In a study of 77 AE patients, *S. aureus* bacterial load was significantly associated with lesionality of the skin (LS vs. NL) but not with FLG mutation (van Mierlo et al. 2022). This is supported by a whole genome sequencing study of healthy and AE participants with and without *S. aureus* colonization, where mutations in

skin barrier genes were not associated with *S. aureus* colonization (Mathias et al. 2015). The overall quantity of the FLG protein is lower in AE (S. J. Brown and McLean 2012), and its breakdown products, urocanic acid (UCA) and pyrrolidone carboxylic acid can impact the density and growth rate of *S. aureus* (Miajlovic et al. 2010). The method by which these molecules impact *S. aureus* is through intracellular acidification (Miajlovic et al. 2010). This highlights that genetic skin barrier dysfunction can influence *S. aureus* colonization, mainly through environmental modulation.

pH can also impact *S. aureus* colonization in AE. *S. aureus* prefers to grow in more alkaline environments (Whiting et al. 1996; Proksch 2018b; Skowron et al. 2021), and pH can impact the effectiveness of skin-native bactericides like Dermcidin where its killing activity was highest at pH 5.5 (Schittek et al. 2001) and β-lactams antibiotics where *S. aureus* are more susceptible to them at acidic pH 5.5 conditions (Lemaire et al. 2007). The amino acid content of AE skin is higher in AE lesions (Ilves et al. 2021; Afghani et al. 2022), and amino acids are known regulators of the buffering capacity of the skin (Levin and Maibach 2008). This coincides with the higher skin pH and reduced buffering capacity in AE patients (Cornbleet and Joseph 1954; Sparavigna, Setaro, and Gualandri 1999; Levin, Friedlander, and Del Rosso 2013b; Panther and Jacob 2015). In addition, in a mouse model, alkalization of the skin resulted in a Th2 response and TSLP-induced scratching (Jang et al. 2016). Although in this study, the mice were not exposed to *S. aureus* (Jang et al. 2016), as explained earlier, aberrant immune system function can benefit *S. aureus* colonization, and alkalization of the skin induced this immune response. A rise in pH within AE coincides with itchiness (Sparavigna, Setaro, and Gualandri 1999; Ali and Yosipovitch 2013), which can be stimulated through *S. aureus*. In addition, *S. aureus* adherence to the stratum corneum is improved at pH 7 compared to pH 5.5 (Miajlovic et al. 2010). Also, as adhesion to a surface is the first step in biofilm formation (Harris, Foster, and Richards 2002), it's unsurprising that biofilm formation within *S. aureus* is pH dependent, where *S. aureus* biofilm formation is best at neutral pH than more acidic or alkaline pHs (Zmantar et al. 2010; Nostro et al. 2012). In summary, there are multiple different pathways at which pH can assist in *S. aureus* colonization in AE.

Alongside pH, oxygen is a currently understudied mechanism that could explain *S. aureus* colonization. *S. aureus* is facultative anaerobic, which means it can grow both in anaerobic and aerobic conditions (Taylor and Unakal 2023). Because segments of the skin can be hypoxic, the scratching of the itchy skin can perforate the barrier turning it more normoxic, and allowing for a unique niche system that *S. aureus* could take advantage of. Still, as of yet,

it has not been studied. Within osteomyelitis, *S. aureus* infection reduces oxygen availability (Wilde et al. 2015). Although unknown in the skin, this activity could benefit *S. aureus* colonization by creating a niche that competing aerobes cannot survive within. Interestingly, when severe AE cases are treated with hyperbaric oxygen therapy, there is a reduction of SCORAD, but this study did not determine if *S. aureus* was present before or after this treatment (Mews et al. 2021). Ozone therapy on AE skin does not impact Staphylococci abundance on LS and NL skin but has been shown to improve microbiome diversity (Zeng et al. 2020). Whether this increase in diversity results in an enrichment of commensal bacteria is still unclear. In addition, as referenced earlier, SCFAs can hinder *S. aureus* colonization, and this production occurs by anaerobic bacteria (den Besten et al. 2013), which could be lost when the skin is severely scratched. Although in opposing directions, oxygen appears to be an essential factor to consider in *S. aureus* colonization and overgrowth in AE.

Lastly, the dysregulation of skin lipids could result in altered *S. aureus* colonization. The levels of sphingosine, a component of the skin's lipid barrier, are reduced in AE (Arikawa et al. 2002). Sphingosine has been shown to negatively correlate with *S. aureus* colonization in AE (Arikawa et al. 2002) and is a known antibacterial against *S. aureus* (Wu et al. 2021). In addition, long chain fatty acids, a component of skin sebum, are reduced in AE (Schäfer and Kragballe 1991; Afghani et al. 2022), and long chain free fatty acids are antibacterial against gram-positive organisms, of which *S. aureus* is a member (Gallo and Nakatsuji 2011). In general, multiple mechanisms can contribute to the colonization of *S. aureus* within AE.

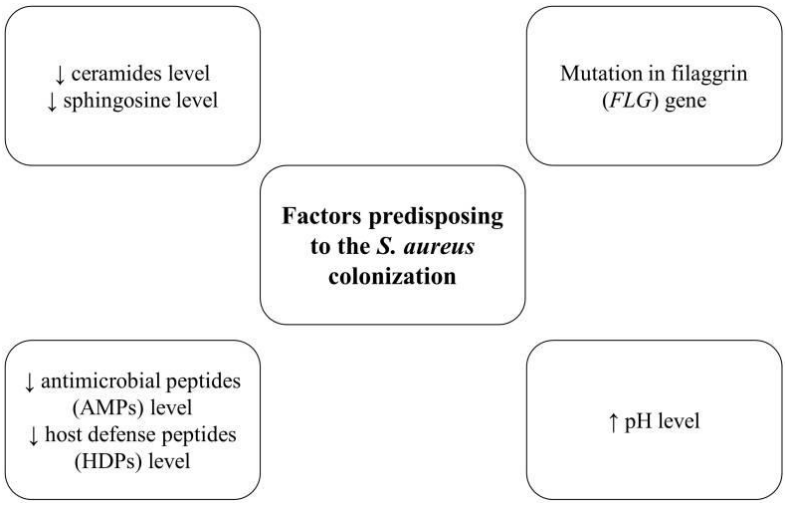


Figure 1.8 Potential Mechanisms of *S. aureus* Colonization

Figure taken from (Ogonowska et al. 2021). There are several observations seen in atopic eczema (AE) that could result in the subsequent colonization of *S. aureus*, such as mutations in the filaggrin gene, rise in skin pH, decrease in antimicrobial peptides and host defensive peptides, and reduction in sphingosine and ceramides.

1.2.5 Strain Diversity in S. aureus

Although the picture of *S. aureus* on the skin suggests that only one version of *S. aureus* colonizes the skin, *S. aureus* has a variety of different strains. For example, *S. aureus* strains from steroid-resistant AE skin have a diverse and larger virulence profile than the general AE population (Schlievert et al. 2008; 2010). In addition, AE strains are heterogenous in toxin production and adhesion genes (Acker et al. 2019). This hints toward potential diversity within AE. As discussed earlier, subsets of the healthy skin population persistently carry *S. aureus* (Armstrong-Esther 1976), and it is estimated that more than 30% of healthy people carry *S. aureus* (Chen, Fischbach, and Belkaid 2018). In mice, healthy *S. aureus* does not elicit the same inflammatory response as AE strains, suggesting strain-specific induction in AE (Byrd et al. 2017). Compared to a laboratory control strain, AE strains encourage T cell proliferation (Iwamoto et al. 2017), and AE strains can penetrate keratinocytes and induce the production of IL-1α through TLR9 (Moriwaki et al. 2019). Of those individuals carrying *S. aureus* in AE, there is only one monoclonal clade on their skin during a disease flare, even though there are differences in which clade when compared across individuals (Byrd et al. 2017). This suggests that one strain of *S. aureus* on the skin rises dramatically during disease flare. Despite the individual heterogeneity, perhaps there is homogeneity regarding why these strains succeed compared to others. AE *S. aureus* strains are highly adaptive to their environment, where steroid treatment generates a niche where *S. aureus* strains respond by secreting more virulence factors (Schlievert et al. 2008). This adaptive ability could extend into other niches like pH and oxygen. In a study comparing three *S. aureus* strains native to bone (naturally hypoxic), although hypoxia resulted in differences in biofilm response, all three had a common mechanistic (gene expression) response to environmental stress (Lamret et al. 2021). These potential group-level (AE vs. healthy) differences have yet to be fully explored in the context of environmental response.

Furthermore, the presence of *S. aureus* on healthy skin contradicts the concept that *S. aureus* is solely pathogenic. Commensal *S. aureus* isolates can produce SCFAs, acetate and butyrate, and when co-cultured with the pathogenic USA300 strain, commensal isolates can reduce pathogenic colonization in wounds (J.-J. Yang et al. 2018). Beyond the direct reduction of pathogenic strains and connecting back to the hygiene hypothesis, priming of the skin's immune system with commensal *S. aureus* followed by later inoculation with a pathogenic strain can reduce the growth of the pathogenic strain by antibody recognition (J.-J. Yang et al. 2018). This highlights the potential use of commensal *S. aureus* as a prophylactic or treatment.

In addition, other compounds, besides SCFAs, may contribute to reduced pathogenic colonization. As far as the researcher is aware, there are no untargeted studies on small molecule secretion-level differences between healthy and AE strain types.

1.2.6 Other Skin Diseases

Although AE will be the main focus of this work, there are many other skin diseases with unique pathogenesis. Two of which are used as disease-specificity controls within this thesis. Chronic spontaneous urticaria (CSU) is an immunological disease with itchy welts on the skin (Kaplan et al. 2023). It is a subtype of chronic urticaria, where the appearance of wheals, angioedema, or both lasts over six weeks with unknown stimuli (Zuberbier et al. 2018). The pathogenesis of CSU is predominantly immunological, where there is mast cell degranulation and histamine release alongside a Th2, Th1/Th17 cytokine profile (Kaplan et al. 2023; Liu et al. 2022). CSU is also associated with autoimmune diseases and pseudo-allergens, and diet-prevention therapy is beneficial (Bansal and Bansal 2019). The gut microbiome has been associated with CSU pathogenesis (Widhiati et al. 2022; Bansal and Bansal 2019), but as of yet, to the author's knowledge, there has been no study on the influence of the skin microbiome. Of what is known for the gut microbiome, there is no consistent microbiome pattern for CSU, with several studies conflicting on the abundance differences for Bacteroidetes and Lactobacilli presence (Widhiati et al. 2022). Based on the current knowledge of CSU, it is predominantly a systemic (i.e., associated with several organ systems dysfunction) immunological disease, and overlaps with AE in regards to the type of inflammation response.

Another skin disease is psoriasis, characterized by epidermis hyperproliferation and keratinocyte cell expansion, resulting in scaly skin (Nikam et al. 2023). Symptoms include itch and pain (Boehncke and Schön 2015). Environmental trigger factors for psoriasis include scratching, chemical irritants (Boehncke and Schön 2015), and smoking (Raharja, Mahil, and Barker 2021). Psoriasis is inherited by over 60% of affected individuals (Dand et al. 2020). Many genes are associated with the immune system or the skin barrier (Raharja, Mahil, and Barker 2021; Quaranta et al. 2014). In contrast to CSU, psoriasis is a collodendritic and T cell-mediated disease where onset is driven by dendritic cell production of TNFα and IL-23 and then T cell continuation where Th17- and Th1-based inflammation occurs (de Rie, Goedkoop, and Bos 2004; Boehncke and Schön 2015; Godlewska et al. 2020). The skin microbiome is suggested to influence psoriasis, but current results are contradictory, and there is no definitive microbiome signature (Godlewska et al. 2020), and the overlap in regards to the skin barrier impairment makes it a good disease-specificity control for AE studies.

1.3 Skin Metabolome

The skin metabolome is the collection of low molecular weight compounds, small molecules ≤ 1.5 kDa, present on and within the skin. Each layer of the skin is home to a unique ecosystem of metabolites. Starting from the deepest layer, the sweat glands are subdivided into eccrine, apocrine, and apoeccrine glands and comprise the sweat metabolome (Sato et al. 1989; Mena-Bravo and Luque de Castro 2014). This segment consists of a myriad of small molecules such as electrolytes like salt, potassium, bicarbonate, and ammonia, urea, lactate, amino acids, and metal ions (Sato et al. 1989). Some of the molecules produced in the sweat glands can be correlated to an individual's plasma or contain amino acids initially originating from the stratum corneum (Sato et al. 1989). Within the skin is also the Interstitial fluid (ISF), a liquid within the connective tissue called the interstitium, composed of water-soluble compounds excreted from the cells and blood capillaries (Wiig and Swartz 2012). Because the ISF is partially composed of compounds secreted from blood capillaries, it unsurprisingly has a high overlap (84%) with metabolites commonly found in the blood (Kool et al. 2007; Niedzwiecki et al. 2018). This metabolome is often non-invasively isolated through the creation of suction blisters, but other methods can also be used (Elpa et al. 2021; Samant et al. 2020). Continuing outward is the stratum basale, and the composition of the stratum basale is mainly phospholipids with the addition of cholesterols and triacyl-glycerides (Feingold and Elias 2014; J. van Smeden et al. 2014). At the stratum granulosum are glucosylceramides, phospholipids and sphingomyelin, all of which are stored in pockets called "lamellar bodies" (Feingold and Elias 2014; J. van Smeden et al. 2014). At the stratum corneum, hydrophilic and lipophilic components produced from the insides and outsides of the corneocytes are found (Bucks 1984). In addition, the stratum corneum contains cholesterols, ceramides, and free fatty acids produced from the breakdown of the lamellar bodies (Elias 1996; Feingold and Elias 2014). These are the metabolites that make up the skin, but the skin can also have metabolites produced from resident microbiota and environmental exposures (Afghani et al. 2022).

A variety of factors can influence the skin metabolome. As our barrier to the outside environment, a variety of environmental factors, UV light, cosmetics, pollution, and the microbiome, have been shown to influence the skin metabolome (Bouslimani et al. 2019; Misra et al. 2021; Randhawa et al. 2014; Patra et al. 2023). Regarding host-related factors, sampling location (Bouslimani et al. 2015) and age (Kuehne et al. 2017) are also influential. The sex of the individual is also suspected to be influential for the skin metabolome, with sweat

metabolomes showing differences (Hooton, Han, and Li 2016). These are the currently known variable factors that can influence skin metabolome results, as recently summarized in (Afghani et al. 2022).

As our outer layer, the skin provides a unique opportunity for monitoring disease. Currently, the skin metabolome can potentially diagnose gut, brain, and lung diseases. In mice, the gut can influence the skin metabolome, where different supplementations, caffeine, green tea, epigallocatechin gallate, and theanine, produce distinct skin metabolomes (Jung et al. 2019). For the brain, the volatile metabolome, the metabolome of the volatile organic compounds (VOCs) which produce odors, of sebum can correctly distinguish Parkinson's disease (Trivedi et al. 2019). This was also confirmed by the sebum's lipidome (Sinclair et al. 2021). Despite this innovation, lipid modeling cannot differentiate between treated and untreated Parkinson's disease (Sinclair et al. 2021). Lastly, the sweat metabolome can not only distinguish lung cancer patients but also hint at the possibility of diagnosing those with risk factors for the development of lung cancer (Calderón-Santiago et al. 2015). Overall, the skin metabolome is a good system for disease monitoring.

1.3.1 Current Sampling Methods

Despite skin metabolomics having the potential for disease monitoring, it is still in its infancy. As hinted at prior, different parts (sebum, sweat, surface) of the skin can be used to measure the metabolome. Despite the skin metabolome being a potential new system for disease diagnosis, there is no standard regarding the sampling method. From the most invasive side, skin biopsies can be taken by skin scrapes or tissue punches. As mentioned earlier, suction blistering can be used for the capture of ISF, but also microneedle patches can be used along with micro-dialysis and open-flow micro-perfusion. With regards to sweat collection, sweating can be stimulated through heating or exercise and then collected. In the case of the macroduct patch, an alkaloid drug that induces sweating is inserted in the skin by an electric field. To forgo this stimulation, one could use a hydrogel micropatch. In addition, for non/minimally-invasive methods not focused solely on sweat collection, tape stripping and pre-wet swabs can be used. This is a brief overview of sampling methods, with a complete overview provided in Figure 1.9 and Table 1.2. Each method for sample collection has pros and cons, described in Table 1.2, and the influential impact of the sampling method has not yet been quantified. Of the methods described among the non-invasive ones, they either take a large amount of clinician, pre- or post-handling time. For example, the current, easiest-to-use non-invasive method, pre-wet swab, requires a pre-wetting and "cleaning" of contaminations for one week

using organic solvents, which makes it unsuitable for clinical settings. More importantly, swabs are not an appropriate sampling method for damaged skin and skin lesions due to the irritation of the rubbing and the drying effect of the solvents, and the total release of the chemicals from the swab after sampling cannot be guaranteed. New sampling methods should be sought because of the need to perform simplistic, cost-effective sampling for metabolomics in the medical and cosmetic sectors.

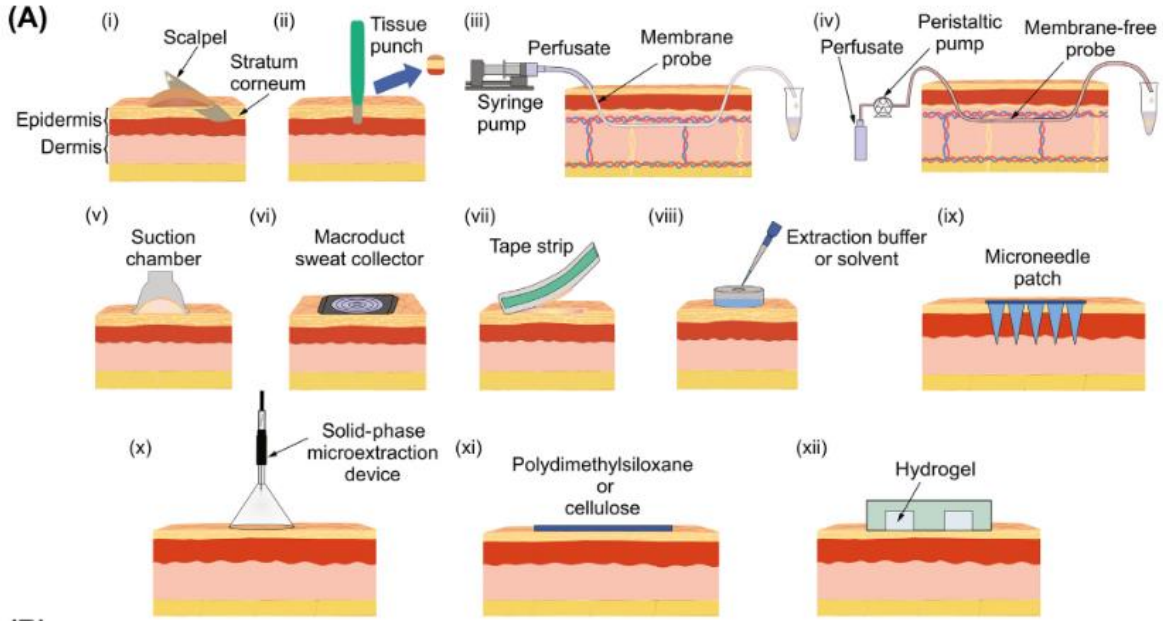


Figure 1.9 Methods for Skin Metabolomic Sampling

Figure taken from (Elpa et al. 2021). Although there has yet to be a comparison of sampling methods for skin metabolome, fourteen different methods have been recorded (only 12 provided here from when the review was published) and tested in the literature. These methods can be highly invasive such as taking sections of the skin with a scalpel, tissue puncture, microdialysis, and open-flow microperfusion; minimally invasive such as skin blistering with a suction chamber, tape strip, microneedle patch; or non-invasive such as collection of the sweat using a macroduct, extraction of the metabolome using a solvent, a cellulose, polydimethylsiloxane, or hydrogel patch, or a solid phase microextraction.

Table 1.2 Pros and Cons of Each Skin Metabolomic Sampling Method

Figure modified from (Dutkiewicz, Chiu, and Urban 2017). Fourteen different methods for skin metabolome sampling including their invasiveness score, sampling time, advantages, and negatives. Abbreviation: no data (n.d.)

Sampling Method	Invasiveness	Sampling time	Pros	Cons
Macroduct	invasive	5 + 30 min 5 + 15 min	several tens of microliters of sweat can be collected	use of drug (pilocarpine) and

				electricity to induce sweating
Solvent Extraction	invasive	~2 min	collection and extraction at the same time	skin irritation
Semipermeable Skin Patch	moderately invasive	0–7 days	enables collection of larger bulk amounts of specimens collected over long periods of time	worn up to a fortnight
			extended time of monitoring	skin irritation, contamination and decomposition of analytes during the prolonged sampling extraction of metabolites from the probe required
Tape Stripping	moderately invasive	~1 min	fast sampling	low specimen load skin irritation after repeated sampling extraction of metabolites from the probe required
Pre-wet swab	moderately invasive	~1 min	fast sampling	extraction of metabolites from the probe required long preparation time
Cotton pad	little or noninvasive	~1 min	fast sampling	extraction of metabolites from the probe required

Textile	little or noninvasive	n.d.	fast sampling	extraction of metabolites from the probe required
Silica plate	little or noninvasive	1 min	fast sampling	low specimen load extraction of metabolites
PDMS film	little or noninvasive	20 min 30 min	trapping volatile analytes	possible decomposition of labile metabolites during thermal desorption
Derivatized silicon	little or noninvasive	<1 min	fast sampling spatially resolved quantification	time-consuming preparation of wafers
Skin blotting with nitrocellulose membrane	little or noninvasive	1–10 min	investigation of protein distribution	antibody-based technique
Oil/microporous membrane	little or noninvasive	2–8 min	nL volumes of sweat can be collected separation of sweat gland excretions from other skin excretions	possible diffusion of lipophilic analytes into oil
Hydrogel micropatch probe	little or noninvasive	1–180 min 20 min	fast sampling online extraction	drying of hydrogel
Micropatch-arrayed pads	little or noninvasive	10 min	fast sampling online extraction Imaging drug distribution on skin	long scan drying of hydrogel

Chapter 1 -Overall Aims of the Thesis

This thesis aimed to deepen the understanding of the micro-environmental factors that influence the skin's homeostasis and develop a simpler method for skin metabolome sampling. The effects of the environmental factors – oxygen and pH - on the microbiome with a special focus on *S. aureus* was explored with a summary found in the Graphical Abstract. As elaborated in the introduction, there is a microbiome present within the skin, and the role of the deeper layer microbiome is still unknown. In addition, currently, the majority of skin microbiome studies measure the relative abundance of bacteria and do not consider actual bacterial load, i.e., absolute abundance. ***(A1)*** I hypothesize that there is a deeper layer microbiome and that the bacterial load differs across the skin layers and within skin disease. In addition, as compositionally, *S. aureus* is commonly overgrowing in AE; ***(A2)*** I expect that there is also a higher absolute abundance of *S. aureus* on the surface of AE skin and due to its capability as an intracellular pathogen, that it penetrates the deep layers of AE skin.

Alongside the presence of bacteria, the low oxygen levels within the skin hypothetically could support an anaerobic microbiome. Since most studies measure the relative abundance of anaerobic bacterial reads or culture the anaerobic bacteria from the skin's surface, it first must be established if there is an anaerobic microbiome within the skin. ***(A3)*** I presume there is an anaerobic microbiome within the skin and, due to the loss of structural proteins like tight junctions and filaggrin and the presence of micro-wounds, ***(A4)*** that the anaerobic microbiome is lost in both LS and NL AE skin.

Also, scratching of the skin not only disturbs the skin but causes excessive contact, potentially resulting in the recolonization of *S. aureus* from other areas of the body. *S. aureus* is facultatively anaerobic and could take advantage of fluctuating oxygen conditions in itchy skin because the skin barrier is damaged from scratching. In addition, *S. aureus* is known to prefer more alkalized environments. Because the pH is increased in AE, and a hallmark symptom of AE is itchiness, ***(B1)*** I suspect that *S. aureus* strains take advantage of this changing ecological niche, where those strains isolated from AE individuals, as compared to healthy isolated strains, are best adapted to AE conditions. Also, *S. aureus* can be found on healthy skin. ***(C1)*** I hypothesize that the secretions of AE and healthy strains differ and that this is environmentally specific.

Along with observing the impact of the environment in vitro, in vivo observations are critical to understanding the effects of the environment on skin health. Physiological measurements are essential to gauge functional changes in the skin, and chemicals, i.e., metabolites, provide a wealth of information about the functional changes to skin homeostasis.

Unfortunately, the current sampling methods for skin metabolomics are not practical for clinical translation due to arduous sample preparation before sampling, the long sampling time required, and the complicated machinery involved. Because of this, ***(D1)*** I aim to develop a new sampling method comparable to the current non-invasive sampling standard, pre-wet swab. From this exploration into the different factors that affect skin homeostasis and the development of more clinically feasible metabolome sampling, we will be one step closer to fully understanding how AE develops and improving how it is diagnosed.

Chapter 2 - Materials and Methods

2.1 Material

2.1.1 Instruments

Table 2.1 Instruments

Instrument	**Supplier**
Sparkreader	Tecan
Illumina MiSeq® platform	Illumina Inc
UPLC-MS^2	Agilent
SICRIT Ionization Source	Plasmion
MS for SICRIT	Thermofisher Scientific
SpeedVac	Fisher Scientific
Sonorex	Bandelin
CFX364 Real Time System Thermocycler	Biorad
Thermomixer F1.5	Eppendorf
Precellys Evolution	Bertin Corp
Whitley DG250 Workstation	Don Whitley Scientific
Centrifuge 5420	Eppendorf

2.1.2 Software

Table 2.2 Software

Software	**Supplier**
CLC Genomics Workbench 11.0.1	Qiagen
Rhea	(Lagkouvardos et al. 2017)
AnnotIEM	(Bhattacharyya et al. 2019)
Spark Control V2.3	Tecan

R	(R Core Team 2022)
Prism version 6.00 for Windows	GraphPad
Microsoft Office	Microsoft
Refiner MS GeneData Expressionist 13.5	GeneData AG
Metaboanalyst v4.0 and 5.0	(Chong, Wishart, and Xia 2019)
MicrobIEM	(Hülpüsch 2021)
MassTRIX	(Suhre and Schmitt-Kopplin 2008)
CFX Maestro Analysis Software	Biorad

2.1.3 Consumables

Table 2.3 Consumables

Consumable	Supplier
96 well plate	Fisher Scientific
Resazurin strips	Schuett-biotec
14mm D-Squame Stripping Discs	Clinical&Derm
Inoculation loop sterile, 10 ul	Carl Roth
Microbank storage vial beads	Pro-Lab Diagnositics
PCR tubes	Eppendorf
qPCR plates 384 well	Thermo Fisher Scientific
Eppendorf Tubes (1.5, 2.0 ml)	Sarstedt
Falcon Tubes (15; 50 mL)	Sarstedt
Screw Cap Eppendorf Tubes (1.5 ml)	Sarstedt
Syringe filter units (0.22 um)	Merck Millipore
Anotox	Don Whitley Scientific
Palladium Catalyst	Don Whitley Scientific
iHILIC®-Fusion UHPLC column, SS, 100 × 2.1 mm, 1.8 µm, 100 Å column	HILICON AB
Glass Vials, LC-MS Certified	Waters
100µm diameter zirconia-silica beads	Carl Roth GmbH + Co

2.1.4 Kits and Reagents

Table 2.4 Kits

Kit	**Supplier**

QIAamp UCP Pathogen Mini	Qiagen
MiSeq® Reagent Kit v3 600 cycles	Illumina Inc
Staphaurex Plus*	Remel

Table 2.5 Reagents

Reagent	**Supplier**
Universal-Silikon	OBI
Resazurin sodium salt	Alfa Aesar
Stool DNA Stabilizer Solution	Stratec
Trypton/Peptone from Casein	Carl Roth
L-cysteine hydrochloride monohydrate	Alfa Aesar
Mannit-NaCl-Agar	Carl Roth
Yeast Extract	Carl Roth
NaCl	Carl Roth
LC-MS grade water	Sigma Aldrich
Acetonitrile, LC-MS grade	Sigma Aldrich
Ethanol, LC-MS grade	Sigma Aldrich
PerfeCTa Multiplex qPCR ToughMix	Quantabio
Stool Stabilizer Solution	Invitek Molecular
Q5 polymerase and reaction mix	New England Biolabs

2.2 Description of Human Studies

In total, five human studies were performed with overlapping techniques.

2.2.1 CK-AD

The data provided from the CK-AD study[5] was only a subgroup of the data with seven healthy (HE) individuals enrolled and three of which had surface and deep layer skin sampling, previously published in (Altunbulakli et al. 2018). For the full study, individuals with clinically diagnosed AE or healthy skin were included, and all ages were accepted. Participants were excluded if they had antibiotic treatment six months before enrollment, and if topical steroids or other treatments were used a month before sampling. Participants were not allowed to use

[5] The CK-AD study is a study that was focused on connecting epidermal barrier dysfunction, as measured through the skin's transcriptome, to microbiome signatures, measured by RNA sequencing in AE and healthy participants. Only three healthy participants in the study had the deeper skin sampled, and therefore, only healthy participants' data will be used for the research within this thesis. The study has been published, see (Altunbulakli et al. 2018).

soaps, sanitizers, skin care products and shower for a minimum of 12 hours (hrs) prior to sampling. The cohort consisted of male and female individuals with ages ranging from 18 to 86. Swab samples were taken from the skin's surface and within the skin's epidermis. The microbiome sampling, sequencing, and data cleaning were performed by prior colleagues listed in (Altunbulakli et al. 2018) at the Institute of Environmental Medicine (IEM). Data analysis is described in section 2.5.

2.2.2 ProRaD

The ProRaD study is a multi-center, time-course observational study focused on AE, described in Bieber et al. (Thomas Bieber et al. 2020) and found at https://ck-care.ch/en/studies/pro-rad-study/. Participants in the study will be observed for a period of five years. The inclusion criteria for this study was individuals with healthy skin and clinically diagnosed atopic disease. The atopic diseases include, but are not limited to the following: food allergy, AE, psoriasis (P), asthma, and allergic rhinitis. There is no age restriction. Use of therapeutics is allowed during the study and is not an exclusion criterion. Patients were excluded when the participant is no longer willing to participate in the study. Data used from this cohort consists of AE - LS (n = 149) and NL (92) - and healthy (HE, 35) samples. Information collected within this cohort ranges from physiological measurements (pH, corneometer, TEWL), severity scoring (SCORAD), bacterial cultivation, microbiome, drug or emollient use, sex, bathing habits/prior bathing, creaming habits/prior creaming, diet, and familial history. Surface swab skin microbiome samples were taken, and the sequencing was performed by other colleagues at the IEM. Data handling and processing is described in section 2.5.

2.2.3 Deep Layer

The Deep Layer study is an observational study of HE, AE, P, and CSU participants. All individuals had to have healthy skin or clinically diagnosed skin disease listed above. Skin-diseased participants were excluded when they did not have lesions at the volar forearm and if they are currently undergoing therapy. The ages of the participants ranged from 21-77. Sampling and data collection were performed by the Metz group within the Department of Dermatology and Allergy Charité at the Universitätsmedizin Berlin. Prior to sampling participants were asked to refrain from using cremes or bathing. A subsection of the individuals who participated in the study had their samples measured by Quantitative PCR (qPCR). Specifically, 28 HE, 14 AE, 5 P, and 17 CSU patients were included. The focus of the study was to observe if skin pH, TEWL, and the microbiome changed at different skin depths (surface

to the stratum basale). These observations were taken by removing layers of the skin with 14 mm D-Squame Stripping Discs, i.e., tape strips (TS) (for more details, see section 2.4). Up to 80 TS were taken per subject. TS2 and TS20 from HE, AE, P, and CSU were used for DNA extraction. For a subgroup of 6 AE and 7 HE, TS40, TS60, and TS80 were used for DNA extraction to observe changes past the stratum corneum, which requires 20 to 50 discs (Jeroen van Smeden et al. 2014). From these samples, the absolute quantity of the microbiome was measured by quantitative polymerase chain reaction (qPCR), described fully in section 2.6.

2.2.4 WET PREP Study (Published)

All protocols described herein were previously published in (Afghani et al. 2021). Only participants without diagnosed skin disease (n=21) or resolved skin disease (n=1) were included in the study.

2.2.5 SICRIT Ionization Source Study

Individuals from the ProRaD study were recruited for a small trial of skin metabolomic sampling. For comparisons between WET PREP and direct skin sampling, three individuals had WET PREP (described in 2.9) sampled and placed their skin directly in front of the SICRIT ionization source attached to a Thermofisher Scientific mass spectrometer (MS) for 30 seconds (sec). The WET PREP samples were heated and placed in front of the SICRIT ionization source to avoid ionization-source-based confounders. In addition, five healthy and three AE individuals' (NL and LS) skin was sampled to determine if the WET PREP could be used for AE diagnostics.

2.3 Description of Bacterial Studies

In addition to human studies, two bacterial studies were performed. The two described in here are the "Time Series Analysis of *Staphylococcus aureus*" (TSASA) and the "Differences in Atopic and Healthy Strain Secretions" (DAHSS) study.

2.3.1 TSASA

For TSASA, see full name above, because of observations of a pH-dependent potential metabolism shift (diauxic shift) within the growth curves of patient strains of *S. aureus* (Figure 2.1), two strains of *S. aureus* (1 HE, 1 AE) were grown over 16 hours in varying pHs (pH 6.0, pH 7.0, and pH 8.0). To cover before, during, and after the diauxic shift, supernatants were collected at three different time points (for details, see section 2.8).

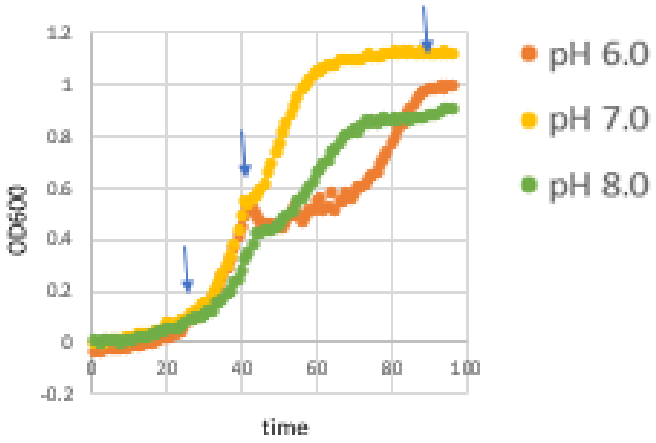


Figure 2.1 Graphical Representation of TSASA Study

Scheme displaying when (blue arrows) during the growth phase of *S. aureus* samples were taken for the TSASA study. The growth pattern displayed is for one strain grown in pH 6.0, pH 7.0, and pH 8.0 media. This figure was generated by Katherine Wald.

2.3.2 DAHSS

Because of the results found from TSASA, a follow up study mimicking the environmental conditions of the AE and HE skin was performed, termed DAHSS. The study design is visually described in Figure 2.2 and Figure 2.3. More specifically, ProRaD bacterial isolates were subjected to four different environmental conditions: aerobic pH 5.5, aerobic pH 7.0, anaerobic pH 7.0, and anaerobic to aerobic flux pH 7.0. These environments were chosen to test normal skin pH -around pH 5.5 - versus the elevated pH in AE - pH 7.0; to test healthy skin oxygen environment -anaerobic; and observe the effects of the scratching conditions – anaerobic to aerobic flux, simply termed "oxygen flux". Anaerobic pH 5.5 was not used as an environmental condition, because the strains were not capable of growth in this condition. Bacterial growth in each condition was measured over 16 hrs, for more information see section 2.7. For a sub-group of the strains, secretions were collected, see section 2.8, and measured by mass spectrometry, see section 2.10. The outline for the number of strains used in the growth curves and secretions can be found in Table 2.6.

Table 2.6 Number of Strains Used for Growth Curves and Measurement of Secretions by UPLC-MS^2

		HE		AE	
	Conditions	**Skin**	**Nose**	**Skin**	**Nose**
Growth Curves	Aerobic, pH 7 Aerobic, pH 5.5 Anaerobic, pH 7	2	13	18	7
	Anaerobic to Aerobic flux	2	13	15	7
UPLC-MS^2	Aerobic, pH 7 Aerobic, pH 5.5 Anaerobic, pH 7	2	13	15	

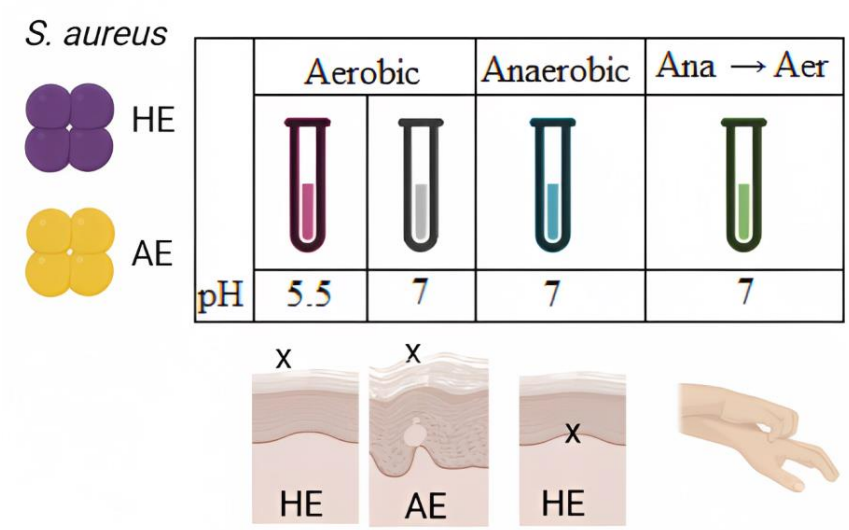


Figure 2.2 Study Design of the DAHSS experiments for Growth Curves

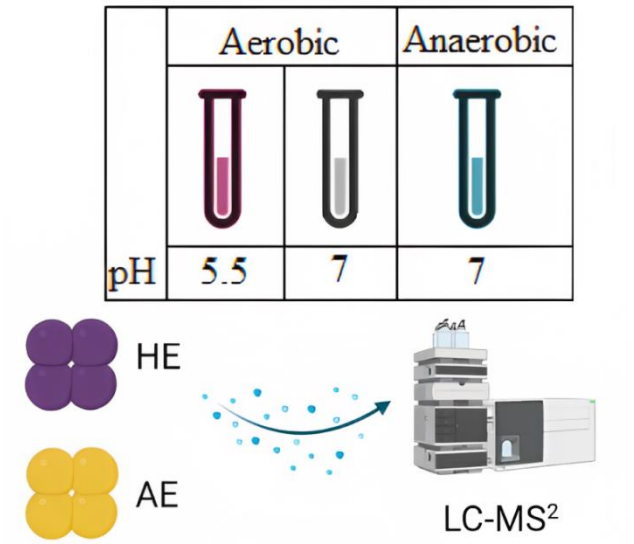


Figure 2.3 Study Design of the DAHSS Study for UPLC-MS^2

2.4 DNA Collection and Extraction

The Deep Layer, ProRaD, and CK-AD study followed different methods for DNA collection. For the Deep Layer study, instead of skin swabs, 14mm D-Squame Stripping Discs (Clinical&Derm) were used because, along with capturing the DNA sequences at that skin layer, they would remove a skin layer, which is a part of the study's goal (to observe changes across the depths of the skin). Discs were taken by applying the disc to the skin, adding a constant pressure with the application tool from Clinical&Derm for 5 sec and then removing with sterile forceps. After sampling, the discs were placed back onto their original foil and stored at -80 °C until thawed. Due to the large size of the D-Squame Stripping Discs, only half of the strip was used for DNA extraction. Immediately before extraction, the discs were cut in half using flame sterile scissors and placed in tubes containing beads (0.5 g, for later bead-beating). For the ProRaD study, skin swabs were collected and stored at -80 °C in tubes with 0.5 g of beads and 500 µL Stool DNA Stabilizer Solution (Stratec). These swabs were later thawed, processed, and measured for 16S rRNA content to determine the bacterial species present in the individuals (HE and AE). Swabs were taken by rubbing the skin approximately 20 times within a region of 2 cm x 2 cm. The CK-AD study followed a similar protocol to the ProRaD study, except that tape strips were used to remove skin layers for the within skin samples, and then a swab sample was taken from the previously stripped area.

All DNA extractions for ProRaD and CK-AD studies were done by other colleagues at the IEM involved in those studies but followed the same protocol, as described below, with minor changes to volumes due to the addition of DNA stabilizing solution. The following protocol was used to extract the Deep Layer study samples' DNA. After sampling, the samples were extracted following the QIAamp UCP Pathogen Mini protocol (Qiagen) for swabs with modifications. In short, 650 uL aliquot of Buffer ATL + Reagent DX was added to the sample; the tubes were then heated at 56 °C for 10 minutes (min) at 0.6 g. Then to release DNA, the samples were bead beaten at 17,664 – 25,436 g for 90 sec, paused for 15 sec, then 17,664 – 25,436 g for 90 sec. Afterward, there was a quick centrifugation of 5 sec at 8,000 g. 400 uL of the supernatant was removed, and to degrade proteins, 40 uL of ProteinaseK was added and heated at 56 °C for 10 min without shaking. After a short centrifugation, 200 uL of Buffer APL2 was added and heated at 70 °C for 10 min without shaking. After another brief centrifugation, 300 uL of ethanol was added, mixed, and centrifuged. The lysate was then transferred to DNA binding columns and vacuumed. To wash the column, 6700 uL APW1 was

added, and a vacuum was applied to remove flow through. Then 750 uL APW2 was added, and the vacuum was used again. Finally, to elute the DNA, 40 uL of Buffer AVE was added to the column, and samples were centrifuged at 20,000 g for 1 min, and this step was repeated once to end up with a total of 80 uL of extracted DNA. Samples were then stored at -80 °C.

2.5 Sequencing and Microbiome Analysis

Both the ProRaD and CK-AD studies had next-generation sequencing performed on their DNA-extracted samples. Sequencing was conducted in collaboration with Klaus Neuhaus at ZIEL Institute for Food and Health (TUM, Weihenstephaner Berg 1, 85354 Freising). The DNA was first quantified by PCR for the V1-3, 16S rRNA gene (primers 27F-YM and 534R). Then 16S rRNA sequencing was done with the Illumina MiSeq® platform (Illumina Inc) and MiSeq® Reagent Kit v3 600 cycles (Illumina Inc). Quality control and operational taxonomic unit (OTU) clustering were done by CLC Genomics Workbench 11.0.1. All species were annotated by the algorithm (AnnotIEM) of Bhattacharyya et al. (Bhattacharyya et al. 2019).

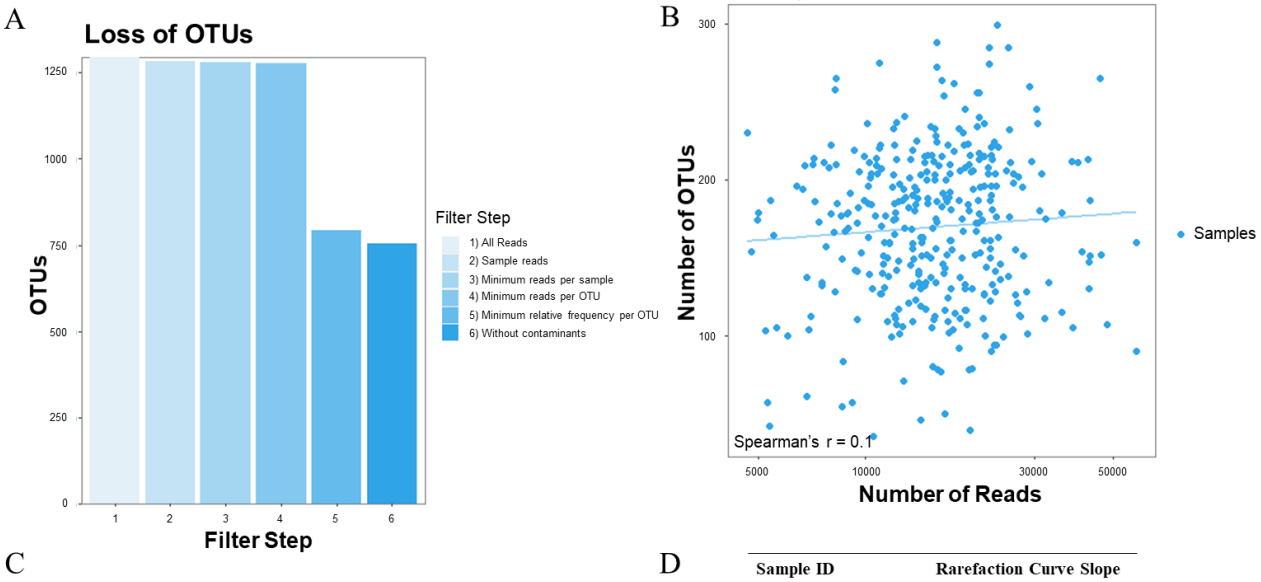
The above steps for ProRaD were done externally by other researchers involved in those studies. For the CK-AD study, all steps except for AnnotIEM were done externally by other researchers involved. The MicrobIEM parameters set for ProRaD can be found in Table 2.7. Afterward, Rhea was used for visualizing the rarefaction curves, and 20 sequences with a high rarefaction curve slope were removed. Information regarding the data's reads and OTUs before and after MicrobIEM filtering and a list of samples excluded after the rarefaction curve can be found in Figure 2.4. After cleaning the data, all microbiome datasets were normalized by dividing each sample by the sum of its total reads.

Table 2.7 MicrobIEM parameters for ProRaD Study

MicrobIEM parameters were set to eliminate contamination and exclude samples with low reads. NEG1 refers to the technical negative controls and NEG2 refers to the biological negative controls.

Parameter	Value
Minimum reads per sample	5000
Minimum reads per OTU	2
Minimum relative frequency per OTU	0.01

Frequency mean ratio (NEG1/SAMPLE)	0.5
Span threshold (NEG1)	75%
Frequency mean ratio (NEG2/SAMPLE)	0.5
Span threshold (NEG2)	50%



Sample ID	Rarefaction Curve Slope
022-ProRad-1-0021	1000
224-ProRad-1-0220	1000
294-ProRad-1-0288	1000
343-ProRad-1-0336	1000
024-ProRad-1-0023	1000
006-ProRad-1-0005	1000
197-ProRad-1-0192	1000
244-ProRad-1-0240	1000
122-ProRad-1-0118	101.247859
112-ProRad-1-0108	94.1806398
130-ProRad-1-0126	79.3308194
246-ProRad-1-0242	78.0490967
328-ProRad-1-0322	54.9953898
096-ProRad-1-0093	52.5716464
021-ProRad-1-0020	47.4593752
077-ProRad-1-0075	42.3305585
116-ProRad-1-0112	41.9252382
309-ProRad-1-0303	41.3103306
075-ProRad-1-0073	39.9684945
319-ProRad-1-0313	39.9467529

Figure 2.4 Filtering and Cleaning of the ProRaD Data

(A) Number of OTUs lost through each step of the MicrobIEM (Hülpüsch 2021) filtering process which is done to remove contaminates commonly present in skin swab samples and to remove low read samples and OTUs. (B) Rarefaction plot of number of reads by number of OTUs per sample after filtering, where the number of reads (x axis) do not strongly correlate with the number of OTUs (y axis). (C) Rarefaction curve of number of reads by number of OTUs after MicrobIEM (red) top 20 under sampled cases. Under sampled cases are determined by the slope of the curve calculated when plotting the number of reads to the number of OTUs (D) Table of rarefaction curve slope for the 20 samples with

The creation of the divisions for anaerobic, aerobic, and facultative anaerobic bacteria, termed the "oxygen tolerance list," was done based on cross-referencing the taxonomic data provided by AnnotIEM to published literature. More specifically, a species' oxygen tolerance was determined based on the information provided by Bergey's Manual. When the information was unavailable in Bergey's Manual, the gen. nov. literature for the species was consulted. If the gen. nov. literature did not specify a species' oxygen tolerance, more recently published literature found through PubMed was consulted. Because of data inconsistencies and the lack of information provided by BacDive, this resource was not used to create the oxygen tolerance

list. This cross-referencing generated four categories: anaerobic, aerobic, facultatively anaerobic, and other. Anaerobic refers to bacteria that cannot survive among atmospheric levels of oxygen. Aerobic refers to bacteria that can survive with atmospheric oxygen. Facultative anaerobic refers to the bacteria that can survive in the presence of atmospheric oxygen and without atmospheric oxygen levels. Other refers to the species of bacteria that currently have no information provided in the literature regarding their survival in relation to oxygen levels, conflicting information provided in the literature, or OTUs that AnnotIEM could not taxonomically identify. The generated list can be found in Supplement Table 7.1 Oxygen Tolerance List.

2.6 qPCR

qPCR was performed on the Deep Layer study samples. The fluorescence-labeled primers were used to measure total microbiome content, 16S rRNA; Staphylococci species, TUF2; *S. aureus*, NUC. Primers were designed by colleagues at IEM (De Tomassi et al. 2023). For primer sequences, see Table 2.8. The concentrations for the primers and probes for the ten times concentrated primer mix is described in Table 2.9. For the volumes of the master mix, the primer mix is used to make the TaqMan MultiProbe see Table 2.10. 5 uL of the TaqMan MultiProbe and 5 uL of extracted DNA were then used for the qPCR. In addition, standard curves created by a serial dilution of the Human gDNA and our pUNIKA-T2 (containing 16S, TUF2, and NUC sequences) were used to determine the number of copies or cells per reaction. The qPCR protocol is to heat the samples for 2 min at 95 ℃ (activation/denaturation), then 45 cycles of 95 ℃ for 15 sec (denaturation) and 60 ℃ for 1 min (annealing and elongation) and was performed on a CFX364 Real Time System Thermocycler (Biorad). The cycle thresholds were calculated from the experimental triplicates using CFX Maestro Analysis Software (Biorad). Experimental triplicates were afterwards averaged to determine the final copies/cells per sample, and AVE buffer was used as a negative control.

Table 2.8 Primer Sequences for Target Genes

Target	Primer Sequence written 5’ to 3’
16S rRNA	Forward: TGGAGCATGTGGTTTAATTCGA
	Reverse: TGCGGGACTTAACCCAACA
	Probe: Cy5-CACGAGCTGACGACARCCATGCA-BHQ2
TUF2	Forward: DCAAATGGACGGMGSTATCT

	Reverse: WGCTGGHACACCAACGTTACG
	Probe: Hex-ATGCCACAAACTCGT-MGB
NUC	Forward: GTTGCTTAGTGTTAACTTTAGTTGTA
	Reverse: AATGTCGCAGGTTCTTTATGTAATTT
	Probe: FAM-AAGTCTAAGTAGCTCAGCAAATGCABHQ1

Table 2.9 10x Concentrated Primers Mix

Primer and Probes	**Concentration (uM)**
hGAPDH	Forward: 2
	Reverse: 2
	Probe: 1
16S rDNA	Forward: 2
	Reverse: 2
	Probe: 1
TUF2	Forward: 8
	Reverse: 4
	Probe: 1
NUC	Forward: 1
	Reverse: 1
	Probe: 1

Table 2.10 Components used to make TaqMan MultiProbe for 1 sample, 5 uL total

Component	uL
PerfeCTa Multiplex qPCR ToughMix	2
Primers mix, 10 times concentrated	1
H_2O	2

2.7 Bacterial Growth Curves

All bacterial strains were isolated from the ProRaD study and confirmed to be *S. aureus* by Mannitol Salt Agar (MSA) appearance and Staphaurex Plus* (Remel). For MSA plate identification, both growth on the plate and a yellow ring surrounding the colony (due to the fermentation of mannitol, which leads to acid production and changes from phenol red, pH indicator, to yellow) denotes *S. aureus* growth. In addition, Staphaurex Plus* - a latex agglutination test determining whether the strain of interest contains a clumping factor, protein A, and surface antigens characteristic of *S. aureus* strains - was used to confirm the species.

Bacterial strains, after isolation from participants in the ProRaD cohort, were stored in bead cryotubes at -70 ℃. These beads were then used to inoculate either Luria Broth (LB) (for TSASA) or modified LB (for DAHSS); a more detailed description can be found in Table 2.11 and Table 2.12. The broth is modified by adding L-cysteine and Resazurin, two components required for the growth and confirmation of growth in anaerobic conditions. The anaerobic and aerobic growth mediums are the same to prevent potential confounding factors from different mediums. The only difference between the two mediums is that the anaerobic medium was degassed in an atmosphere of 5% H, 85% N, and 10% CO_2 following autoclaving and the aerobic medium was not. For both TSASA and DAHSS, the bacterial strains were first inoculated overnight at 37 ℃. Afterward, their OD_{600} was measured, and dilutions required for OD_{600} = 0.01 were calculated. They were then diluted for the desired OD_{600} (0.01) and pipetted into a 96-well plate, and grown, shaking by continuous double orbital (amplitude 1 mm, frequency 270 rpm) at 32 ℃ for 16 hrs with OD_{600} measured every 10 min by a Sparkreader (Tecan). The Sparkreader read the OD_{600} with ten flashes and a settling time of 50 ms. Multiple reads were taken per well in a circle filled with a 1500 um border 2x2 orientation.

Table 2.11 Ingredients used to make 1L of Luria Broth
After dissolving ingredients in water, the solution was pH adjusted according to the desired pH.

Component	Amount (g)
Tryptone	10

NaCl	10
Yeast Extract	5

Table 2.12 Ingredients used to make 1L of modified Luria Broth
After dissolving ingredients in water, the solution was pH adjusted according to the desired pH.

Component	Amount (g)
Tryptone	10
NaCl	10
Yeast Extract	5
L-cysteine HCl x H_2O	0.5
Resazurin Solution (0.0436 Molar)	100 uL

When grown in anaerobic conditions, the method is slightly modified. The media used was at pH 7.0. All strains were inoculated and diluted under anaerobic conditions (5% H, 85% N, 10% CO_2). The color of the resazurin solution indicates confirmation of anaerobic conditions, where yellow LB indicates anaerobic conditions maintained and pink LB indicates the presence of oxygen. The anaerobicity of the chamber was also confirmed before inoculation by resazurin strips (Schuett-biotec). After diluting into the 96-well plate and prior to removal from the anaerobic chamber, the 96-well plate was sealed with a silicon paste (OBI) to retain the anaerobic conditions within the plate while being grown and measured in the Tecan Sparkreader. Based on prior literature, silicon was used as a sealant (Koutny and Zaoralkova 2005). The steps taken for anaerobic conditions were repeated for the anaerobic to aerobic, i.e., oxygen flux environmental condition, except that the 96-well plate was not sealed before it was removed from the anaerobic chamber.

Following measurement, the OD_{600} measurements are averaged across the experimental triplicates (3 wells) or for anaerobic samples duplicates (2 wells) and subtracted from the blank OD_{600} measurement (LB-only wells). Growth curves were created with Microsoft (MS) Office, and the area under the curves was calculated with GraphPad Prism version 6.00 for Windows.

2.8 Collection of Bacterial Supernatant

Bacterial supernatant was collected in the following studies (1) TSASA and (2) DAHSS. For the first study, biological duplicates and experimental replicates of two strains of *S. aureus* (1 HE, 1 AE) were grown at three different pHs (pH 6.0, pH 7.0, and pH 8.0) in LB media (described in Figure 2.1). At 3 hrs, 6 hrs and 40 min, and 16 hrs, 150 uL of the bacteria were

harvested and centrifuged at 1000 g for 10 min at 4 °C. 100 uL of supernatant was removed and snap frozen by immersion in liquid nitrogen. Samples with less than 100 uL possible for recovery were excluded from analysis (3 samples: S32_AD_0047_497_pH7_6 hr 40 min., S47_ HE_9014_322_pH7_6 hr 40 min., and S71_ HE_9014_322_pH7_16 hr). In parallel, 150 uL aliquots of uninoculated media were processed similarly. These samples were then stored at -80 °C until processed and run on UPLC-MS^2 (see section 2.10).

For the second study, biological triplicates of 30 strains of *S. aureus* (15 HE, 15 AE) were grown in three conditions (described in Figure 2.3) in modified LB media. After 16 hrs, 900 uL of the bacteria were harvested and centrifuged at 1000 g for 10 min at 4 °C. 700 uL of supernatant was removed and snap-frozen by immersion in liquid nitrogen. In parallel, 900 uL aliquots of uninoculated media were processed similarly. These samples were then stored at -80 °C until processed and run on UPLC-MS^2 (see section 2.10).

2.9 Collection of WET PREP and Pre-wet Swabs

Skin metabolome samples were collected using WET PREP or a pre-wet swab at the antecubital fossa. This protocol can be found in (Afghani et al. 2021). Briefly, WET PREP consists of a 5 mL water lavage, where 1 mL increments were collected. After sampling, the samples were centrifuged at 847 g for 10 min. Pre-wet swab sampling followed the protocol described in (Bouslimani et al. 2015), where swabs were incubated in a 500 uL 50/50 solution of water and ethanol for a week, with the solution being exchanged every 2nd day. After sampling, the swab was incubated for 2 hrs at 4 °C, vortexed, and filtered (0.22 um). All metabolomic samples were snap frozen after processing and stored at -80 °C until run on UPLC-MS^2.

2.10 Mass Spectrometry

UPLC-MS^2 (Agilent) was performed in the WET PREP, TSASA, and DAHSS studies. WET PREP followed a similar protocol described below with modifications on the volumes, and the in-detail protocol can be found in (Afghani et al. 2021). All samples were thawed once before processing to prevent the loss of metabolites by multiple freeze-thaw cycles. Supernatants were first evaporated in a SpeedVac vacuum concentrator set to 35 °C and 1 mbar. The pellets were then resolved in 100 uL of LC-MS grade water (Sigma-Aldrich). To precipitate out proteins, 900 uL of cold LC-MS grade Acetonitrile (Sigma-Aldrich) was used with centrifugation (15 minutes 4 °C, 21,000 g). The supernatant (950 uL for TSASA, 915 uL

for DAHSS) was collected and then dehydrated in the same conditions described previously. The TSASA samples were then resolved in 50 uL of an equal mix of acetonitrile and water. Because of the focus on polar metabolites for the DAHSS study, a higher acetonitrile concentration (90% acetonitrile, 10 % water; 50 uL per sample) was used to resolve the samples. For both TSASA and DAHSS studies, 5 uL of the sample was pooled for quality control (QC). Samples were randomized before injection to reduce the impact of variation due to injection order. To measure consistency in column equilibration and stability across the run, before the run, ten QC injections were run, and to ensure consistent stability, for every ten samples, a QC was injected. The injection volume was set to 5 uL. During the run, the samples were kept at 4 ℃. Electrospray ionization was used to ionize the samples. For both studies, an iHILIC®-Fusion UHPLC column, SS, 100 × 2.1 mm, 1.8 μm, 100 Å column (HILICON AB) was used with samples run in the negative electrospray mode. MS parameters were set according to previously published literature (Sillner et al. 2019). Briefly, the column was kept at 40 ℃, and the run time was 12.5 min per sample with a flow rate set to 0.5 mL/min. Mobile phase A consisted of 95% acetonitrile/5% water and 5 mM ammonium acetate, and mobile phase B consisted of 30% acetonitrile/70% water with 25 mM ammonium acetate. The first 2 min mobile phase B was at 0.1%, then increased to 99.9% over 7.5 min. This percentage was kept for 2.5 min and decreased to 0.1% within 0.1 min.

The raw data was processed in Refiner MS GeneData Expressionist 13.5 (Genedata AG) with the assistance of Constanze Müller at Research Unit Analytical BioGeoChemistry (HMGU). For TSASA, both the sampling and methodological blanks were subtracted with a two-fold and five-fold threshold. Before statistical analysis, the samples were minimum value imputed and normalized by the number of bacterial cells calculated from the OD_{600}. Any further log transformation, median normalization, and autoscaling(mean-centered and divided by the standard deviation of each variable) were performed by Metaboanalyst v4.0 and 5.0 (Chong, Wishart, and Xia 2019). For DAHSS, the methodological blanks were subtracted with a 5-fold threshold, and all other steps were the same, except the data were mean-centered instead of autoscaling as for TSASA. All clusters were putatively annotated, as defined by (Sumner et al. 2007; Viant et al. 2017), by MassTRIX (Suhre and Schmitt-Kopplin 2008).

2.11 Statistical Analysis

For statistical significance, the following tests were utilized and described in Table 2.13. Statistical significance is defined as two-tailed with at least $p \leq 0.05$, and the stars

symbolism is described in Table 2.14. All p-values were corrected for multiple testing for the mass spectrometry data according to the Benjamin and Hochberg false discovery rate. After multiple testing (Kruskal-Wallis or Friedman) was performed, a post-hoc test for multiple comparisons (Dunn's or Wilcox matched pairs signed rank) was performed. Correlations were done with the Spearman rank test. In addition, for the DAHSS study, ASCA is the combination of ANOVA with PCA, where PCA is performed on an effects matrix designed according to experimental design. Statistical analysis and the plots were created with R (R Core Team 2022), GraphPad Prism version 6.00 for Windows, and Metaboanalyst 4.0 and 5.0 (Chong, Wishart, and Xia 2019).

Table 2.13 Description of Statistical Tests Used

Test	Parametric/ Non-parametric	Continuous/ Categorical	Paired/Non-Paired	Post-hoc Test	Number of Groups
Mann-Whitney	Non-parametric	Continuous	Non-paired	No	≤ 2
Wilcox matched pairs signed rank	Non-parametric	Continuous	Paired	Yes	≤ 2
Dunn's Test	Non-parametric	Continuous	Non-paired	Yes	≤ 2
Kruskal-Wallis	Non-parametric	Continuous	Non-paired	No	> 2
Friedman	Non-parametric	Continuous	Paired	No	> 2
Spearman rank	Non-parametric	Continuous	Non-paired	No	N.A.

Table 2.14 Symbols for Significance and Their Meanings

Symbol	Meaning
ns	$P > 0.05$
*	$P \leq 0.05$
**	$P \leq 0.01$
***	$P \leq 0.001$
****	$P \leq 0.0001$

Chapter 3 - Results

3.1 Observations of Skin Microbiome Across Depth

The skin is composed of multiple layers, with a differential oxygen concentration across those layers. This difference in oxygen concentration can influence the presence and composition of the microbiome because different species respond differently to oxygen levels. In this section, a macroscopic look at the influence of oxygen on the microbiome is explored.

3.1.1 Absolute Quantification of Microbiome at the Different Skin Depths

Because skin depth should reflect the decreasing oxygen concentration in the skin (surface to deep skin), this section aimed to observe the influence of skin depth on the absolute abundance of the microbiome. The qPCR results shown here were generated from the Deep Layer study, where TS were collected from the same location repetitively to capture the different layers of the skin. The Deep Layer study included AE, psoriasis (P), CSU, and HE participants, and the number of participants in each group can be found in Figure 3.1.

To begin with, the microbiome was not consistently detected in all samples. No copies of 16S were detected at the surface (TS2) of the skin for 29% HE, 36% AE LS, and 36% AE NL samples (Figure 3.1). In contrast, 16S copies were detected in all samples at TS2 for P LS, P NL, and CSU (Figure 3.1). At TS20, more 16S copies were detected for HE, going from 71% to 79% of samples, and the deeper the TS went, the more samples had 16S copies, except for a drop at TS60, where only 86% of samples had 16S copies detected (Figure 3.1). For AE NL, the trend of the deeper the TS went, the more samples had 16S copies was also seen, except for a drop at TS80, where 83% of samples had detectable 16S (Figure 3.1). For AE LS at TS20, there was a decrease in the percentage of samples that had detectable 16S from 64% to 50% (Figure 3.1). This percentage then increased again as one went deeper down the layers (Figure 3.1). At TS20 for P NL, there was no decrease for samples with detectable bacteria, but for P

LS, the number of samples with detectable 16S copies decreased to 80% (Figure 3.1). Finally, for CSU at TS20, 88% of the samples had 16S copies (Figure 3.1). Although not all samples had a detectable microbiome, most samples had detectable 16S copies by qPCR.

Of those that had detectable 16S copy numbers, the quantity of the bacteria decreases with increasing skin depth regardless of disease status (Figure 3.1). The steepness of this downward slope for bacteria quantity differs between HE and AE (Figure 3.1). For HE, there appears to be one large drop in 16S copies at the TS20, while for AE LS, there are two drops – the first at TS20 and then at TS60 (Figure 3.1). AE LS samples also have this two-drop pattern (Figure 3.1).

Along with a decrease in bacterial abundance, between the disease statuses, the absolute quantity of the skin's bacterial load differs (Figure 3.1). In descending order, the amount of 16S copies at the skin's surface (TS2) are AE LS and P LS, AE NL and P NL, HE, and CSU (Supplement Figure 7.1). This order of bacterial quantity is the same within TS20 (Supplement Figure 7.1). Significant differences exist in bacterial abundance between HE and AE and HE and P LS samples. There is a non-significant difference in 16S copies between HE and CSU for TS2 and TS20 (Supplement Figure 7.1). At TS20, the range of 16S copies for both AE NL and P NL skin shrinks considerably (Supplement Figure 7.1). In addition to TS2 and TS20 for a subset of the HE and AE individuals, qPCR was also performed on TS40, TS60, and TS80. Although visually different at these skin depths, no significant difference was found between HE and AE samples and AE LS and AE NL (Supplement Figure 7.1).

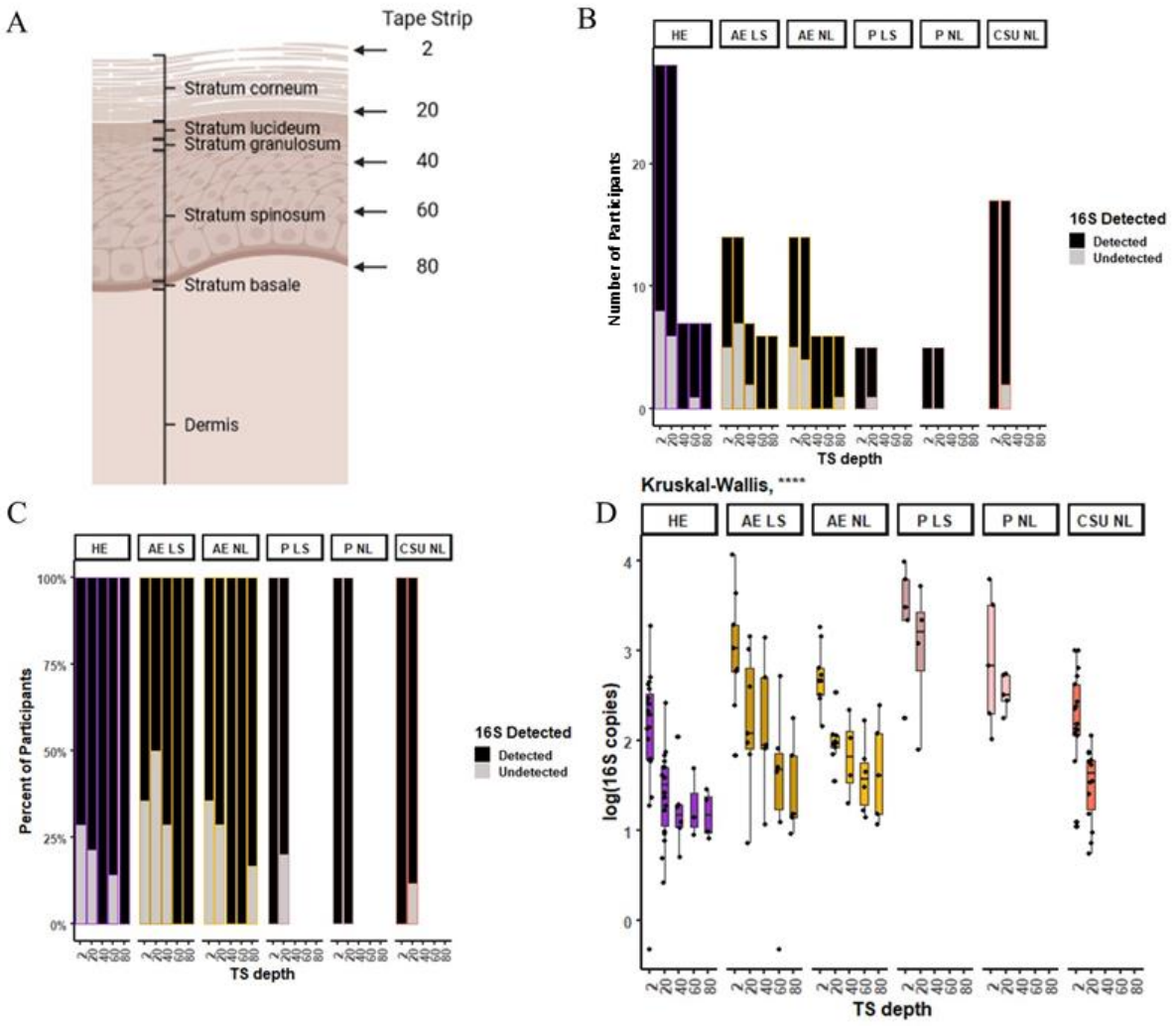


Figure 3.1 Detection of Absolute Quantity of Microbiome

(A) Approximate location of tape strips within healthy skin according to literature (Jeroen van Smeden et al. 2014; Olesen et al. 2019; Sølberg et al. 2018). (B) Total number of participant samples in the study per health status and tape strip (TS) depth with number of samples that have 16S detected in black and undetected in grey. (C) Stacked bar chart of the percentage of participants with 16S detected (black) and 16S undetected (grey) according to TS depth and health status. (D) Boxplot with overlaid dot plot of the log(16S copies) according to health status and TS depth. Samples with undetected 16S copies were removed and 16S copies were subtracted from background copies (copies detected in an empty tape strip). Each dot represents one sample. Color scheme and Abbreviations: Psoriasis lesional (P LS; dark pink), Psoriasis non-lesional (P NL, light pink), Chronic Spontaneous Urticaria (CSU, coral), Atopic Eczema non-lesional (AE NL, yellow), Atopic Eczema lesional (AE LS, dark yellow), healthy (HE, purple).

3.1.2 Absolute Quantification of Staphylococci and S. aureus at the Different Skin Depths

Along with looking at the abundance of bacteria across the skin depths, a more microscopic look at the influence of oxygen on the microbiome is explored within select bacteria. Staphylococci are known skin colonizers and are made up of both commensal and pathogenic species; one such skin pathogen is *S. aureus* which is known to be overgrown in

AE skin. Currently, there is no knowledge as to whether the quantity of Staphylococci and *S. aureus* differs across the skin depths. Here, we observed the abundance of Staphylococci and *S. aureus* across the skin depths and in relation to disease severity.

Across all health statuses, Staphylococci cells are present, which decreases with increasing depth, with two exceptions: from TS20 to TS40 in AE LS samples and after TS20 in AE NL samples (Figure 3.2). The number of Staphylococci cells increase in AE LS samples from TS20 to TS40. In addition, for AE NL samples, the amount stabilizes after TS2 (Figure 3.2). The abundance of Staphylococci cells is comparable across both HE and CSU for TS2 and TS20 (Supplement Figure 7.2). Relative to healthy, there is a significant rise in cells for AE LS and P LS (TS2 and TS20). For P NL, the cell increase is only significant at TS20 (Supplement Figure 7.2). *S. aureus* was only seen in AE samples where they were present as far down as TS40 in AE LS. The infiltration of *S. aureus* down the layers of the skin for AE NL only went as far as TS2, where there were minuscule levels at TS20 (Figure 3.2).

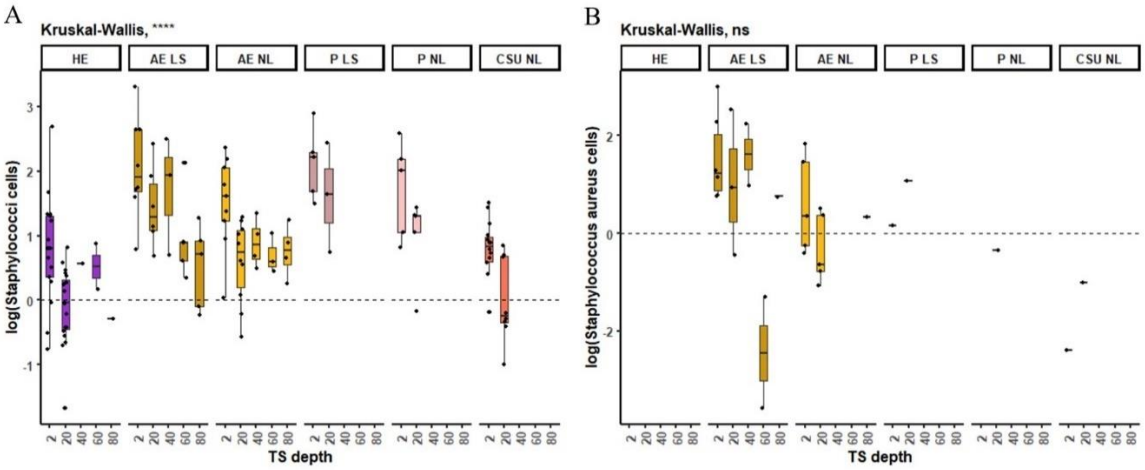


Figure 3.2 Staphylococci Abundance across the Skin Depths

(A) Boxplot with overlapped dot plot of the log(Staphylococci cells) according to health status and tape strip (TS) depth. Each dot represents one sample. Samples with undetected 16S reads, and Staphylococci cells were removed. Staphylococci cells are calculated according to the TUF2 copy number, with one TUF2 copy per cell. (B) Boxplot with overlapped dot plot of the log(*S. aureus* cells) according to health status and tape strip depth. *S. aureus* cells are calculated according to NUC copy number, where there is one NUC copy per cell. Color scheme and Abbreviations: Psoriasis lesional (P LS; dark pink), Psoriasis non-lesional (P NL, light pink), Chronic Spontaneous Urticaria (CSU, coral), Atopic Eczema non-lesional (AE NL, yellow), Atopic Eczema lesional (AE LS, dark yellow), healthy (HE, purple).

Because there was a more prominent presence of Staphylococci in diseased skin relative to HE, the correlation between Staphylococci cells and severity was measured. For AE, only the local EASI was significantly negatively correlated to the number of Staphylococci cells at

TS2 (R = -0.71) (Figure 3.3). For P NL, at TS20, there was a significant positive correlation (R=1) (Supplement Figure 7.3). All other correlations were not significant.

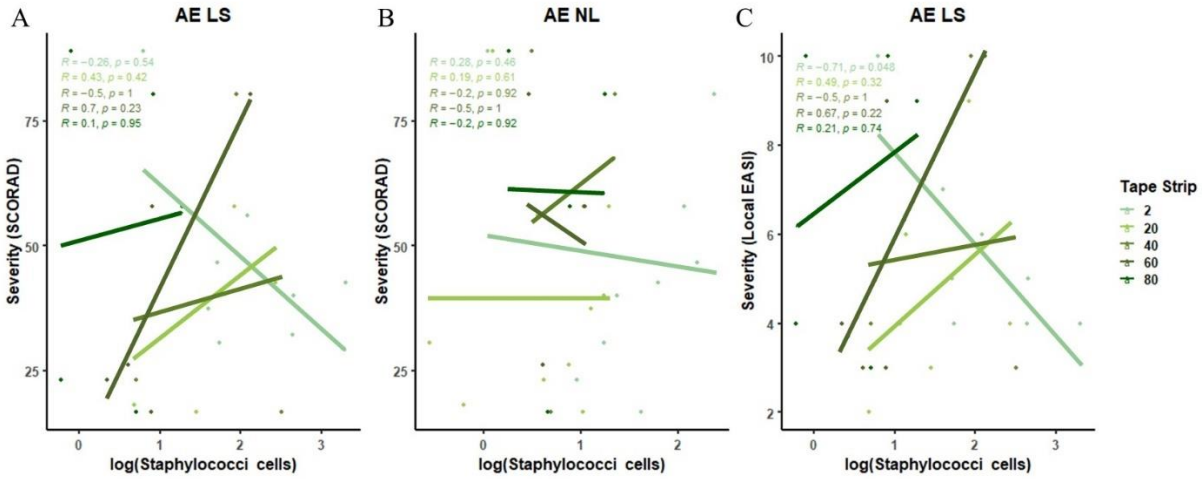


Figure 3.3 Correlation of Severity to Staphylococci in Atopic Eczema

All severity measures taken were correlated to the log of the Staphylococci cells, and each dot represented a sample. (A) AE LS samples where the severity measure was SCORAD Index (B) AE NL, SCORAD Index (C) AE LS, local EASI. Data was stratified according to tape strip number. All data was stratified by Tape Strip: TS2 (darkseagreen3), TS20 (darkolivegreen3), TS40 (darkolivegreen4), TS60 (darkolivegreen), TS80 (darkgreen).

3.2 The Anaerobic Microbiome's Relationship to Skin Depth and Disease

3.2.1 Influence of Skin Layer on Anaerobic Microbiome

As there is a differential oxygen concentration across the skin layers, it was hypothesized there would also be a subsequent variable anaerobic bacteria abundance. To test this, the CK-AD study was performed. Microbiome samples were collected from seven healthy individuals, with three individuals providing microbiome samples from the deeper layers of the skin. The taxonomy of the CK-AD study was then categorized into aerobic, anaerobic and facultative anaerobic and "Other"; for more details, see section 2.2.1 and 2.5. In general, the taxa were evenly divided among the four categories (Figure 3.4). At the skin's surface, the relative frequency of anaerobic bacteria was strongly individually dependent, with one individual having no anaerobic bacteria on the skin (Figure 3.4). When averaging across the individuals, the aerobic microbiome has the most drastic change and is larger in the deep layer of skin when compared to the surface (Figure 3.4). On the other hand, the average anaerobic microbiome does not appear to change, but pairwise comparisons were subsequently performed because of its highly individualistic nature (Figure 3.4). There is a general trend of higher relative abundance of anaerobic bacteria in the deeper layers, but this is only valid for two of the three individuals sampled (Figure 3.4).

A

Oxygen Tolerance Label	Percentage of OTUs
Aerobic	25
Anaerobic	30
Facultative Anaerobic	22
Other	23

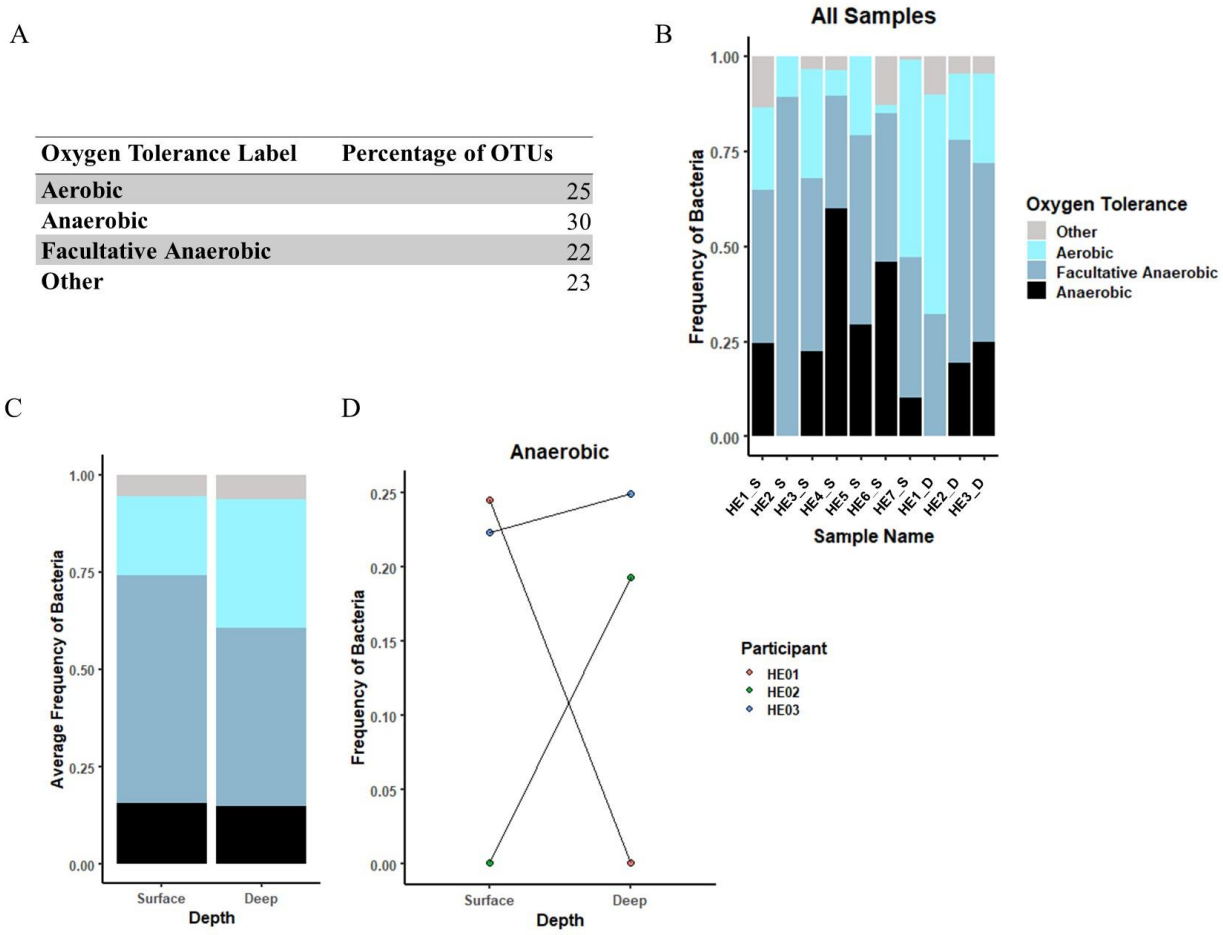


Figure 3.4 Oxygen Tolerance Within Surface and Deep Layer Skin

Figures from analysis of CK-AD data where surface and deeper layer skin samples were taken from healthy individuals. (A) Percentage of OTUs (bacteria) labeled into each oxygen tolerance, independent of abundance of the OTUs within the samples. (B) Relative abundance of anaerobic OTUs across all samples. Healthy surface samples are indicated by "_S" and deep samples by "_D". (C) Average relative abundance across paired samples according the skin sampling depth. Color scheme for (B) and (C): other (grey), aerobic (bright blue), facultative anaerobic (dull blue), anaerobic (black). (D) Relative abundance across paired samples comparing the frequency of bacteria according to skin sampling depth; healthy participant 1 (HE01, pink), HE02 (green), HE03 (blue).

3.2.2 Impact of Anaerobic Microbiome in Atopic Eczema

In addition to observing the anaerobic microbiome within healthy individuals, one must observe and compare with imbalanced skin to better understand skin homeostasis. If anaerobic bacteria are important for the skin's homeostasis, then changes in this microbiome should be observed in imbalanced, diseased skin. This section aimed to examine the relative abundance of the anaerobic microbiome within healthy skin compared to AE. The ProRaD dataset was used; more details are found in section 2.2.2. Although ProRaD data only collects microbiome samples from the skin's surface, it can further deepen the knowledge of the anaerobic microbiome and its importance in the context of AE.

As seen in the CK-AD study, the relative abundance of the anaerobic microbiome appears to vary across individuals (Supplement Figure 7.4). For the AE samples, there is an inverse relationship, where with decreasing anaerobic frequency, there is an increase in aerobic frequency (Supplement Figure 7.4). This trend is not as clearly seen in the healthy samples (Supplement Figure 7.4). Regarding average frequency, the average frequency of anaerobes is decreased in AE, both NL and LS, with a subsequent increase in the facultative anaerobic average frequency (Figure 3.5). Taken singularly, the anaerobic microbiome frequency is significantly higher in HE samples when compared to AE LS (Figure 3.5), and the inverse is true for the facultative anaerobic frequency (Supplement Figure 7.5). There is no significant difference between the frequency of anaerobic bacteria between AE LS and NL samples (Figure 3.5).

To determine confounding factors, two methods were used: (1) separately to view the individual influence in relation to the frequency of anaerobic bacteria and (2) in concurrence to view the combined influence in relation to distinguishing health status with step-wise regression. For the first part, bathing and skin treatment within 12 hrs of sampling did not influence the frequency of anaerobic bacteria (Supplement Figure 7.7). In addition, visit number, patient's age, and sex did not affect the frequency (Supplement Figure 7.6, Supplement Figure 7.7). Microenvironment appears to influence anaerobic frequency, with dry skin having slightly more anaerobic bacteria, but this is not significantly different to sebaceous and moist skin (Supplement Figure 7.6).

Regarding the second part, the data was first divided into two groups (a) HE and AE LS and (b) HE and AE NL. Afterward, the best-fit model based on forward and backward regression was determined for each group. Only potential confounding factors that could directly impact the skin's microbiome were taken to prevent over-fitting. Those factors are microenvironment, skin treatment, bathing, and health status. For LS skin, no confounding factors impact the differentiation seen for anaerobic frequency between healthy and AE skin (Supplement Figure 7.8). For NL skin, the moist microenvironment and use of skin treatment are confounding factors that impact the anaerobic frequency observed in healthy versus AE skin (Supplement Figure 7.8).

The SCORAD (Scoring Atopic Dermatitis) index, a severity scoring measurement system for AE, has a significant ($p = 0.026$) albeit low correlation ($R = -0.27$) to the frequency of anaerobic bacteria in NL samples (Figure 3.5) with an even lower correlation ($R = -0.18$, $p = 0.04$) within LS samples. To parse out if the frequency of anaerobic bacteria is distinct across

the levels of severity, the samples were categorized according to grades of severity- mild, moderate, and severe- based on cut-offs determined by (Pucci et al. 2000). The frequency of anaerobic bacteria was found to be significantly different between mild and severe AE with a general decrease in the median from mild to moderate to severe (Supplement Figure 7.6). Although select species within the anaerobic microbiome could predominantly contribute to the reduction in frequency between AE and HE and between the two severity grades of AE, there is a 91% overlap between the OTUs found in HE, AE LS, and AE NL samples (Figure 3.5).

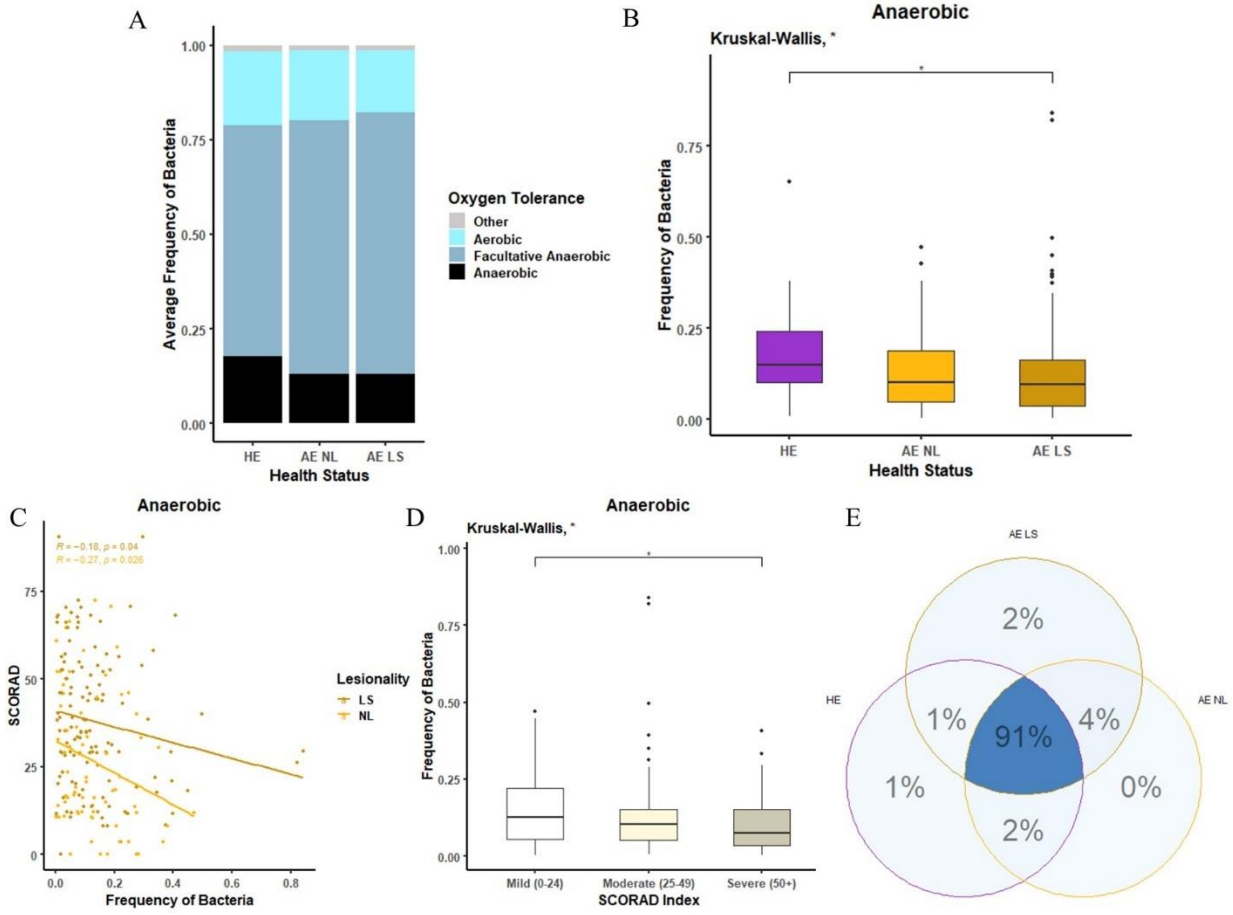


Figure 3.5 Anaerobic Microbiome in ProRaD

(A) Average frequency of Anaerobic OTUs across all samples separated according to health status. Frequency was calculated by dividing the relative abundance by the total counts of reads. Other (grey), aerobic (bright blue), facultative anaerobic (dull blue), anaerobic (black). (B) Relative abundance of anaerobic OTUs across HE (n=35, purple), AE NL (92, yellow), and AE LS (149, burnt yellow) samples. Significance determined by Kruskal-Wallis with Dunn's Test as post-hoc analysis. (C) Correlation of frequency of anaerobic OTUs to SCORAD severity. R and p-value calculated by Spearman's rank correlation coefficient. (D) Relative abundance according to total SCORAD severity groups: mild (n= 76, white), moderate (77, light yellow), or severe (52, tan) groups. Significance determined by Kruskal-Wallis with Dunn's Test as post-hoc analysis. (E) Venn Diagram of anaerobic OTUs detected in HE, AE LS, and/or AE NL samples. Abbreviations: Atopic Eczema non-lesional (AE NL), Atopic Eczema lesional (AE LS), healthy (HE).

Chapter 3 -Environmental Influences on Staphylococcus aureus Behavior in Relation to Atopic Eczema

3.3 Environmental Influences on *Staphylococcus aureus* Behavior in Relation to Atopic Eczema

Although oxygen has been interpreted as an important factor in skin homeostasis, it is not the only environmental factor impacting it. The pH significantly correlates to the microbial abundance at the skin's surface (TS2) and significantly and strongly correlates to the presence of Staphylococci (Figure 3.6). In addition, as seen in the Deep Layer study, *S. aureus* is specifically present within AE (Figure 3.2). Additionally, facultative anaerobes are capable of growth in anaerobic and aerobic conditions, and the scratching of the skin could change the oxygen gradient within the skin and subsequently modulate bacterial growth. Therefore, *S. aureus* abundance, pH, and scratching play essential roles in skin and disease development and must be observed in conjunction with oxygen to best reflect the skin environment in vitro.

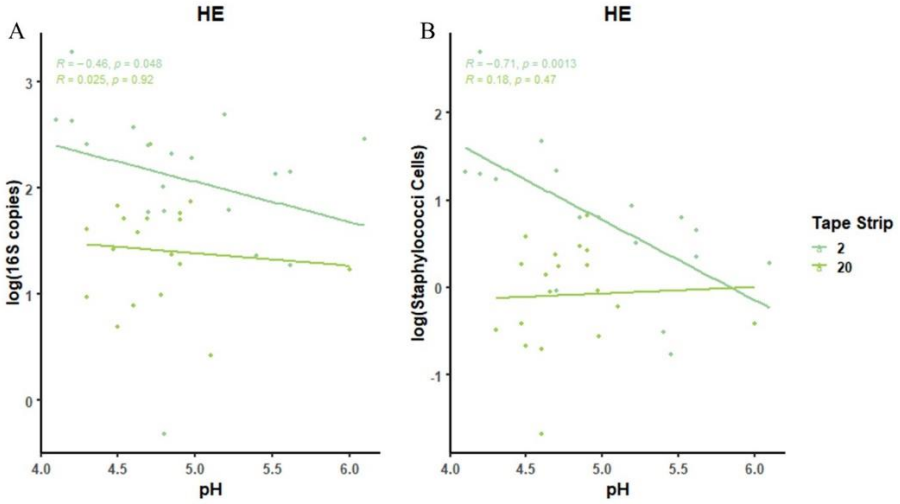


Figure 3.6 Relationship of pH to Absolute Bacterial Load

(A) Correlation of Skin pH to the log of 16S copies. (B) Correlation of Skin pH to the log of Staphylococci cells. R and p-value calculated by Spearman's rank correlation coefficient. All data was stratified by Tape Strip: TS2 (darkseagreen3), TS20 (darkolivegreen3). Data taken from Deep Layer study.

Because *S. aureus* can interact with the chemical environmental factors of the skin, these interactions were simulated in vitro to gain a deeper understanding of how the abiotic influences the biotic within AE. *S. aureus* strains isolated from healthy (HE) and AE participants' nose or skin. Their strains were grown in four representative environments: aerobic pH 5.5 (healthy surface skin), aerobic pH 7.0 (AE skin), anaerobic pH 7.0 (healthy deep skin), and anaerobic to aerobic flux pH 7.0 (scratching skin). The area under the curve (AUC) was calculated to compare across the strains, with a higher AUC representing better "fitness" to the growth environment. When observed individually, there is a range of AUCs across the strains (Supplement Figure 7.9), especially in maximum potential growth condition

(pH 7.0, aerobic). To better account for this variability and improve comparisons, pH 7.0, aerobic, was set as a baseline, with all other AUCs subtracted from this environment's AUC within each strain, creating ΔAUC. For ease of interpretation, higher positive numbers would mean a more drastic difference to the baseline, lower positive numbers denote less of a baseline difference, and negative numbers would mean that the strains grew better than the baseline. Individually, the oxygen difference (ΔO_2) has the highest ΔAUC, followed by the pH difference (ΔpH) and oxygen flux difference (ΔO_2 flux) (Figure 3.7). No significance was found between the comparison of ΔpH and ΔO_2 flux; both result in minimal inhibition of growth compared to baseline (Figure 3.7).

After stratifying according to participant health status, ΔpH had no significant change between the groups (Figure 3.7). Both oxygen and oxygen flux differences had significant differences between HE and AE strains, with higher ΔAUC (Figure 3.7). Within AE, nose and skin isolates are not different according to ΔAUC (Figure 3.7). Healthy nose isolates have significantly higher ΔAUC than AE skin isolates (Figure 3.7). For ΔO_2 flux, there is a significantly higher ΔAUC for AE skin compared to AE nose, indicating location as a confounding factor (Figure 3.7). Despite that, when compared within the same isolation location (nose), HE isolates have significantly higher ΔAUC than AE isolates (Figure 3.7). The general trend within ΔO_2 flux is a decreasing ΔAUC from HE skin to HE nose and a further decrease from AE skin and AE nose (Figure 3.7). Overall, the environment impacts the fitness of *S. aureus*, with the level of impact differing due to environmental conditions, participant's skin health status, and isolation location.

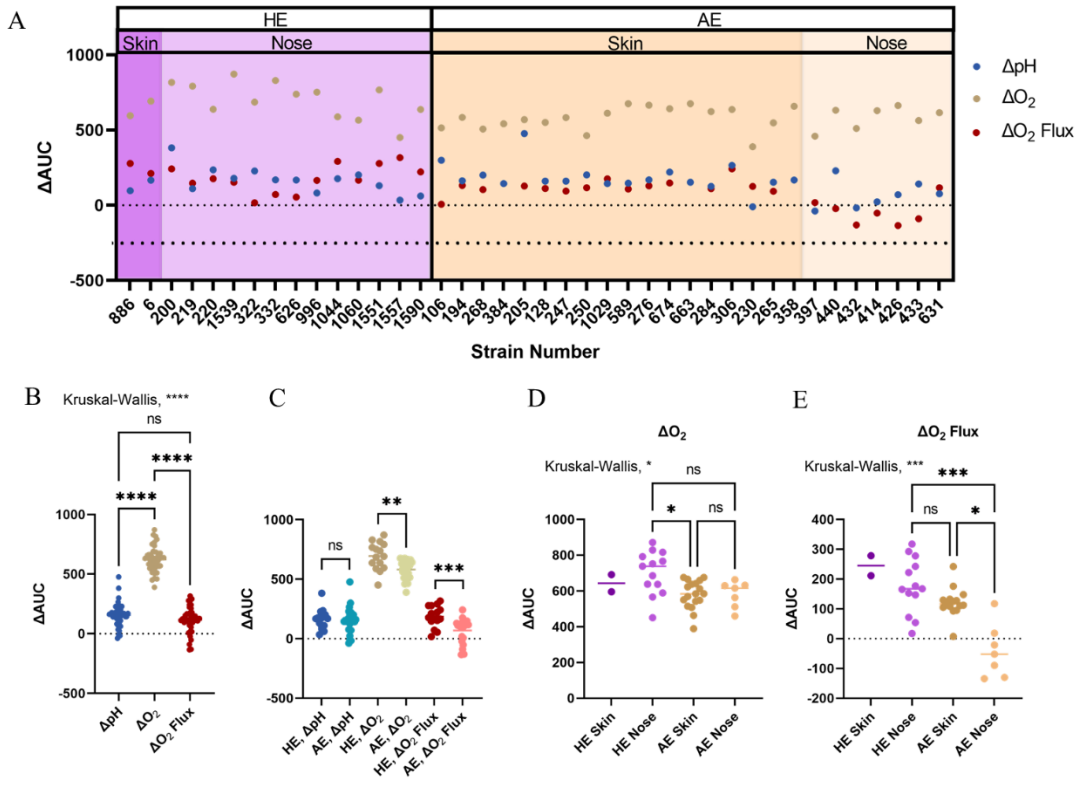


Figure 3.7 Comparison of Difference in Area Under the Curve (ΔAUC) across pH, Oxygen, and Oxygen Flux Environments

AUCs for aerobic, pH 5.5; anaerobic, pH 7; or oxygen flux were subtracted from Aerobic, pH 7 (for example Aerobic, pH 7 – Aerobic, pH 5.5) to generate the ΔAUC (A) ΔAUC for each *S. aureus* strain and colored according to environment grown: ΔpH (blue), ΔO_2 (tan), ΔO_2 Flux (red). Purple background indicates healthy (HE) strains (purple background), atopic eczema (AE) strains (yellow background), skin isolates (darker background shade), nose isolates (lighter background shades).(B) Comparison of across ΔAUC environments. (C) Stratification between healthy strains (darker shades) and atopic eczema strains (lighter shades) within environments. Mann-Whitney test, not corrected for multiple testing (D) Within ΔO_2 environment, the ΔAUC separated by location of isolation. (E) Within ΔO_2 Flux environment, the ΔAUC separated by location of isolation. For (B), (D), and (E) a post-hoc Dunn's test was performed, and for (D) and (E): skin (darker shades) and nose (lighter shades).

3.4 Environmental Influences on *Staphylococcus aureus* Secretions in Relation to Atopic Eczema

Although bacterial abundance is important to skin health, this merely reflects its role in providing skin-influencing chemicals from its secretions. Therefore, to improve the understanding of the role of *S. aureus* in the skin, one must naturally explore their secretions. Because our disease of interest is AE, where the skin has altered oxygen and pH conditions, these conditions were simulated in vitro. Overall, this section aims to determine the effects of the abiotic factors (oxygen and pH) on *S. aureus* metabolite secretions.

3.4.1 Impact of Microbial Stages of Growth on S. aureus Secretions

Before testing all the in vitro skin environments, a preliminary study, TSASA, was performed. This study aimed to determine if metabolites were found in the secretions of *S. aureus*, along with deciding at which growth stage the secretions should be collected. In addition, to obtain an initial glimpse of whether the environment can impact *S. aureus* secretions, the strains were grown in three different pH environments (pH 6, pH 7, and pH 8). Timepoint 180 min was the beginning of the exponential phase, 400 min was the middle of the exponential phase, and 960 min was the stationary phase.

The growth stages had a large impact on the metabolite secretions. The average number of metabolites between the two *S. aureus* strains was highest at the stationary phase (Figure 3.8). Based on PCA, the stationary phase cluster is separated from the other two growth phases and has a large distribution across the principal components (Figure 3.8). This distribution is due to the pH of the growth medium, where pH 6, pH 7, and pH 8 form their distinct clusters (Figure 3.8). This segregation based on pH is not as clearly seen in the secretions from the beginning and middle of the exponential phase (Figure 3.8). In summary, the growth stage and environment impact *S. aureus* metabolite secretions.

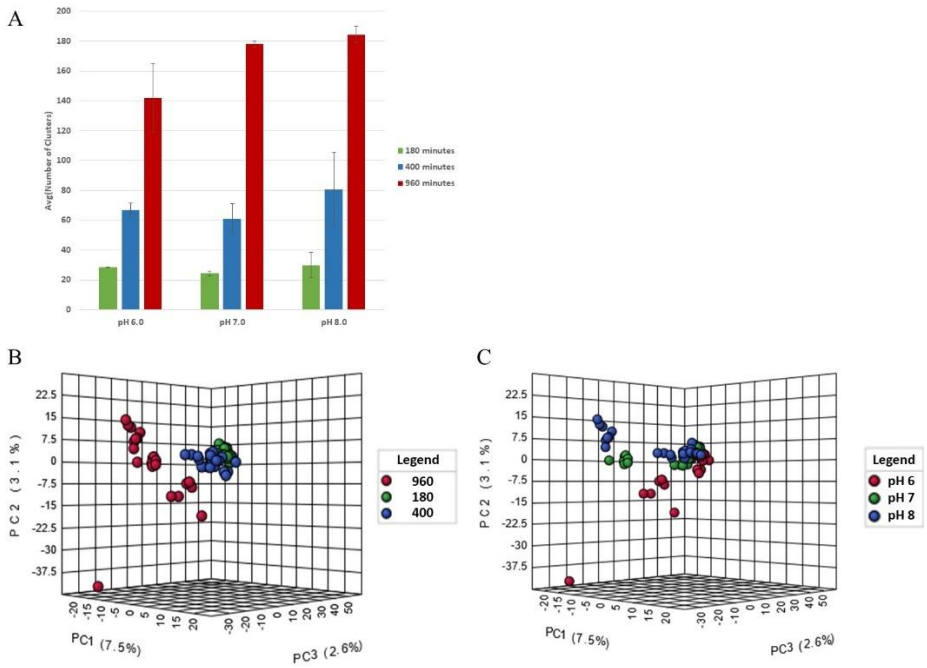


Figure 3.8 Influence of Growth Stage on Metabolite Secretion

(A) Average number of metabolites present in the samples according to length of time the strains were grown from starting 0.01 OD_{600} and within each pH environment (pH 6.0, 7.0, and 8.0). Error calculated by standard deviation. Color scheme: 180 minutes (green), 400 minutes (blue), 960 minutes (red). (B) Principal component analysis (PCA) of samples colored according to time (C) PCA of samples colored according to their pH growth environment. For (B) and (C) three principal components are shown with their percentage of explained variance.

3.4.2 Predominate Environmental Influence on S. aureus Secretions.

This chapter mirrors section 3.3 but excludes the oxygen flux conditions. Because of the results from section 3.4.1, only the secretions at 960 min were measured to get the largest number of metabolites and best view of the environmental impact. Both HE and AE strain secretions are compared across and within the growth environments to deepen the understanding of the influence of the environment on *S. aureus* secretions in the skin and to gauge if HE and AE strains respond differently to different environments. The conditions *S. aureus* grew in were selected to represent the different health statuses of the skin, and a schematic of these conditions is provided in Figure 2.3.

To begin with, the secretions predominately separate according to growth conditions (Figure 3.9). The two aerobic conditions cluster closest to each other (Figure 3.9), so oxygen seems to be a predominate segregation factor, with pH being the second distinguishing factor (Figure 3.9). Within each PCA cluster, there is no clear initial separation of HE and AE strains (Figure 3.9).

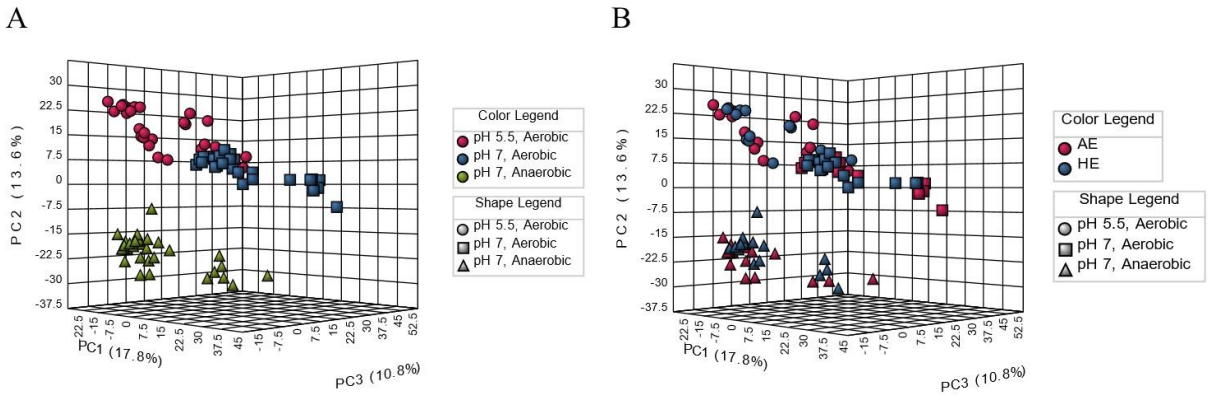


Figure 3.9 Overall Segregation of Secretome by Environment

(A) Principle component analysis (PCA) of *S. aureus* strains colored according to growth environment: pH 5.5, aerobic (red); pH 7.0, aerobic (blue); pH 7.0, anaerobic (green). (B) PCA of strains colored according to strains' health status: AE (red), HE (blue). Each strain has three biological duplicates that were averaged across to create one data point per strain. The principal components are shown with their percentage of explained variance.

To understand the trends between the interaction of health status and their environment and select the relevant metabolites involved, ANOVA simultaneous component analysis (ASCA) was performed. First, a scree-test was performed to determine which component would be used to model the data, where the component that can describe the highest amount of variation was chosen. Metabolomic variation induced by the interaction of the environment and health status could not be well modeled by the data (Supplement Figure 7.10). The variation could be accurately modeled only when the factors (growth environment and health status) are separate (Supplement Figure 7.10).

Because of the ASCA interaction results between the environment and the strain's health status, the data was then separated into each health status to parse out the finer details regarding the influence of the environment on the secretions of both healthy and AE strains. As visible through the PCA, the environment highly influences the secretions for both HE and AE strains (Figure 3.10). In addition to the environmental clustering, there is also a separation within each environment that is not specific to select strains and cannot be explained by the isolation location (Figure 3.10). In congruence with the environment score plot by ASCA, according to the dendrogram, the secretions of AE and HE strains grown in pH 5.5, aerobic and pH 7.0, anaerobic conditions are more similar to each other than pH 7.0, aerobic (Figure 3.10, Supplement Figure 7.10).

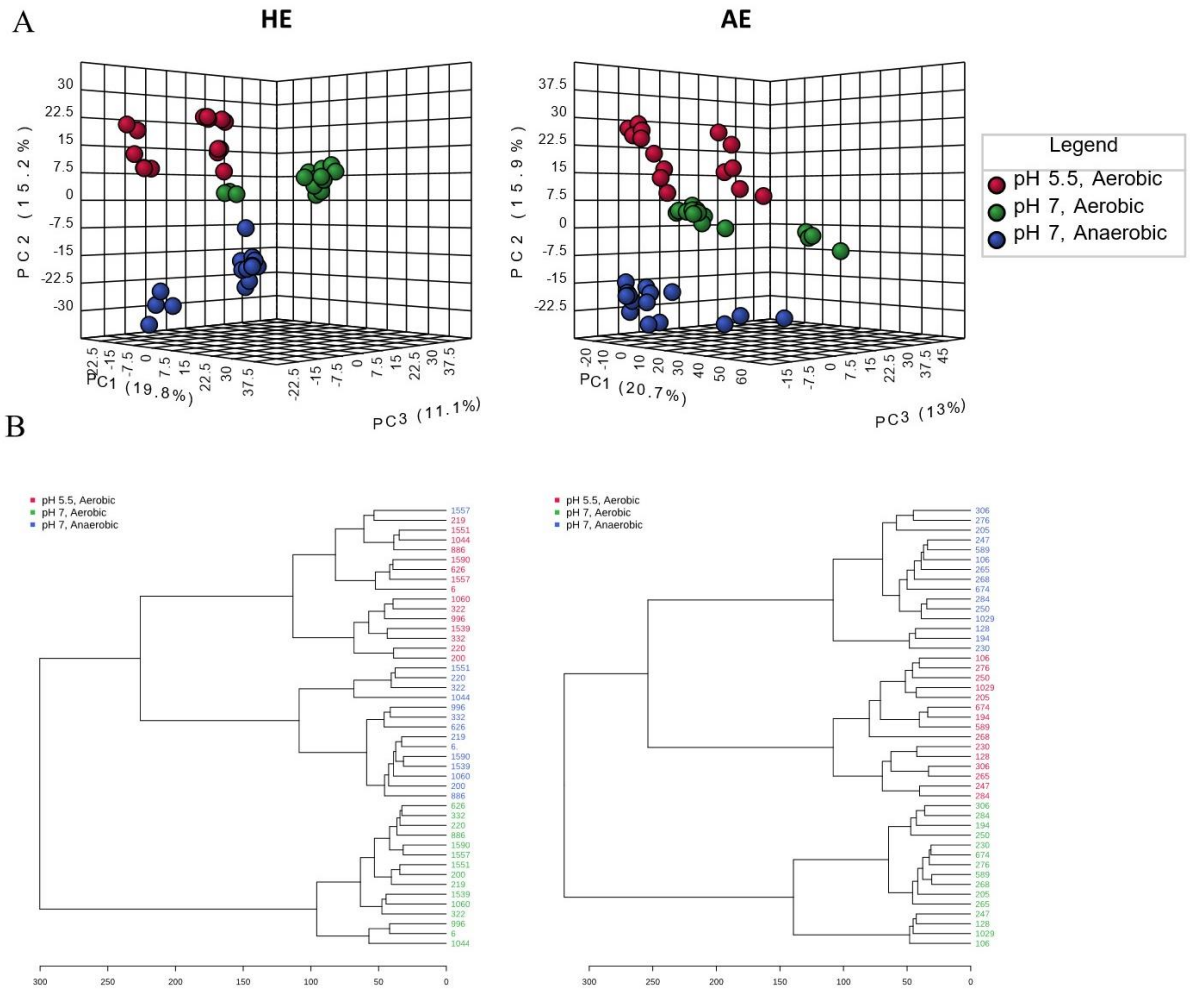


Figure 3.10 Differences across the Environments Within Each Health Group

(A) Principal component analysis of strains stratified by health status, healthy (HE, left) and atopic eczema (AE, right), and colored according to growth environment: pH 5.5, aerobic (red); pH 7.0, aerobic (green); pH 7.0, anaerobic (blue). (B) Dendrogram with distance measurement Euclidian and cluster algorithm Ward of the same samples where they are stratified by health status, HE (left) and AE (right) with coloring according to growth enviornment.

Looking deeper into the environmental differences within each group (HE, AE), significantly different metabolites across the environments were determined according to a volcano plot with limits $p \leq 0.05$ and a fold change higher than 5-fold. These significantly different metabolites found through comparison of pH 5.5, aerobic versus pH 7.0, aerobic ($s\Delta pH$) and pH 7.0, aerobic versus pH 7.0, anaerobic ($s\Delta O_2$) were then compared to each other to determine if the bacteria's response to the environment is the same regardless of what the environmental perturbation is. From the Venn diagrams in HE, only 25% of the significantly different metabolites change regardless if it is a pH or oxygen change (Figure 3.11). 36% of

the significantly different metabolites are specific to the changes in the oxygen environment, and 39% are specific to pH changes (Figure 3.11). For AE strain secretions, of the 27% of the metabolites that differ regardless of the environment perturbation, 36% are specific to $s\Delta O_2$, and 36% are specific to sΔpH (Figure 3.11).

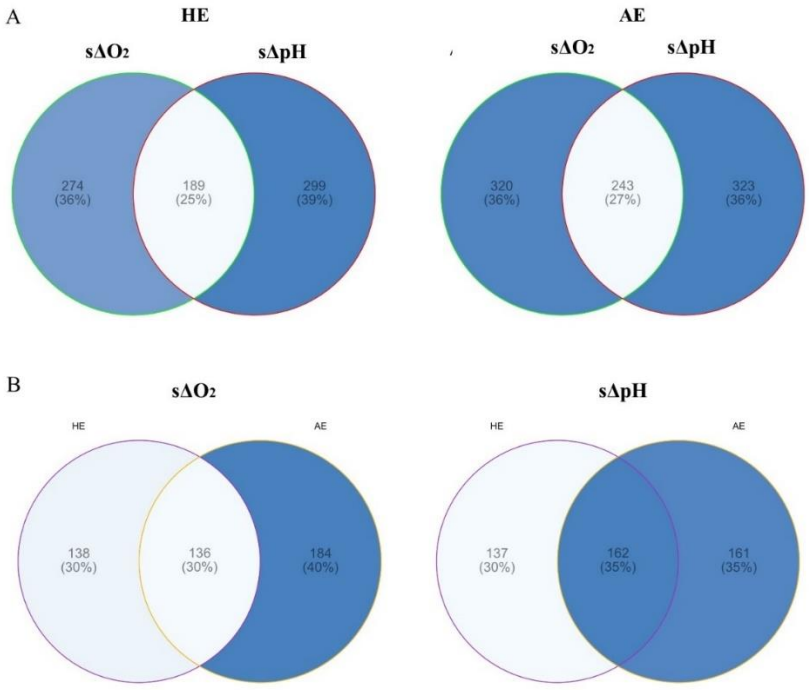


Figure 3.11 Overlap of the Significantly Different Metabolites across Environments for HE and AE Strains

Most significant different features across the environments were determined by volcano plot with limits $p \leq 0.05$ and folder change higher than 5-fold. (A) Venn diagram of the number of significantly different features between environments pH 7.0, aerobic versus pH 7.0, anaerobic (sΔO2) and pH 5.5, aerobic versus pH 7.0, aerobic (sΔpH) within healthy (HE) and atopic eczema (AE) strains (B) Venn diagram showing the overlap of unique features, determined by the Venn Diagram in (A), for environments $s\Delta O_2$ (right) and sΔpH (left) where features are compared between, HE and AE strains. Numbers correspond to the number of features with percentage corresponding to the percentage of overlap and unique features within the Venn diagram.

Along with the strains' secretion response to the environment within HE or AE strains, it is also interesting to understand whether the different metabolite secretion responses across the environments are the same between HE and AE strains. The unique metabolites specific to sΔpH or $s\Delta O_2$ were compared between the HE and AE groups. For $s\Delta O_2$, 30% of the metabolites overlap across HE and AE strains; for sΔpH, it is 35% (Figure 3.11). Most metabolites that uniquely respond to $s\Delta O_2$ or sΔpH are specific to HE or AE strains (Figure 3.11).

Chapter 3 -Environmental Influences on Staphylococcus aureus Secretions in Relation to Atopic Eczema

Besides focusing on the bacteria's response to environmental changes, the difference in the secretions between HE and AE strains within each growth environment is explored. Among pH 5.5, aerobic no clearly defined clusters formed among HE and AE strains (Figure 3.12). Despite this, significantly different metabolites were found, with the majority of these metabolites being secreted more by AE strains (Figure 3.12). In contrast, once the pH became 7.0, regardless of the oxygen environment, there was clear AE and HE strains clustering. Most of the significantly different metabolites between HE and AE within each environment could not be tentatively annotated after cross-reference to the KEGG/HMDI/LipidMaps databases by MassTRIX (Figure 3.12). For those that could be tentatively identified, only acetic acid could be found within the skin - as derivative amino acetic acid - after comparison to the data collected in the WET-PREP study (Afghani et al. 2021)(Supplement Table 7.2, Supplement Table 7.3, Supplement Table 7.4). The intensities for the significantly different metabolites, across HE versus AE, are similar across the strains for the majority of the significantly different metabolites (Figure 3.13). The secretion differences within each environment are also mainly unique to each environment, as seen in the Venn diagram (Figure 3.13). Only three could be tentatively annotated for the eight clusters that overlap between aerobic environments (pH 5.5 and pH 7.0) (Figure 3.13). These are Acetyl-blasticidin S, PD123177, and adonitoxin (Supplement Table 7.2, Supplement Table 7.3). Although many of the metabolites could not be identified, it is clear that the environment influences the secretions of *S. aureus* differently between HE and AE strains.

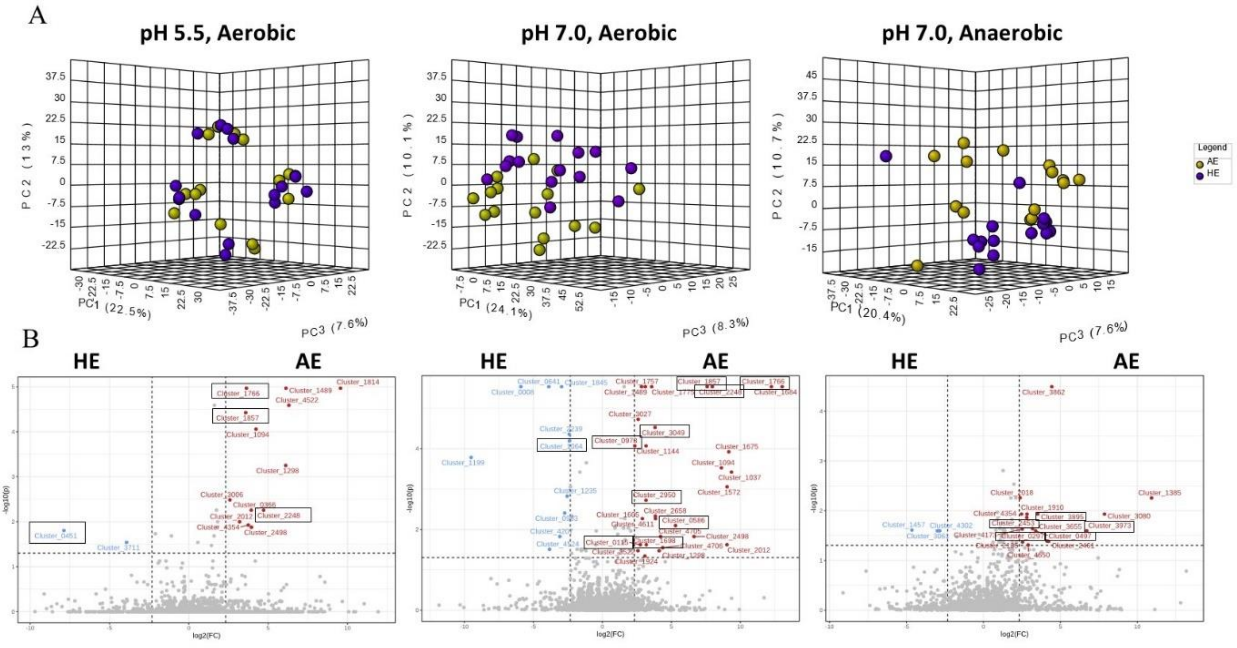


Figure 3.12 Secretion Differences within Each Growth Condition

(A) Principal Component analysis (PCA) where strain secretions are separated according to each environment: pH 5.5, aerobic (left); pH 7.0, aerobic (middle); pH 7.0, anaerobic (right). The samples are colored according the health status - healthy (HE) and atopic eczema (AE). (B) Volcano plot between health statuses (HE vs AE) and separated according to each environment with limits $p \leq 0.05$ and fold change higher than 5-fold. Color scheme: Metabolites found more in healthy samples (blue), metabolites found more in atopic eczema samples (red), and nonsignificant clusters (grey). Black boxes denote that this metabolite has been identified by MassTRIX with a 0.005 Da cut-off.

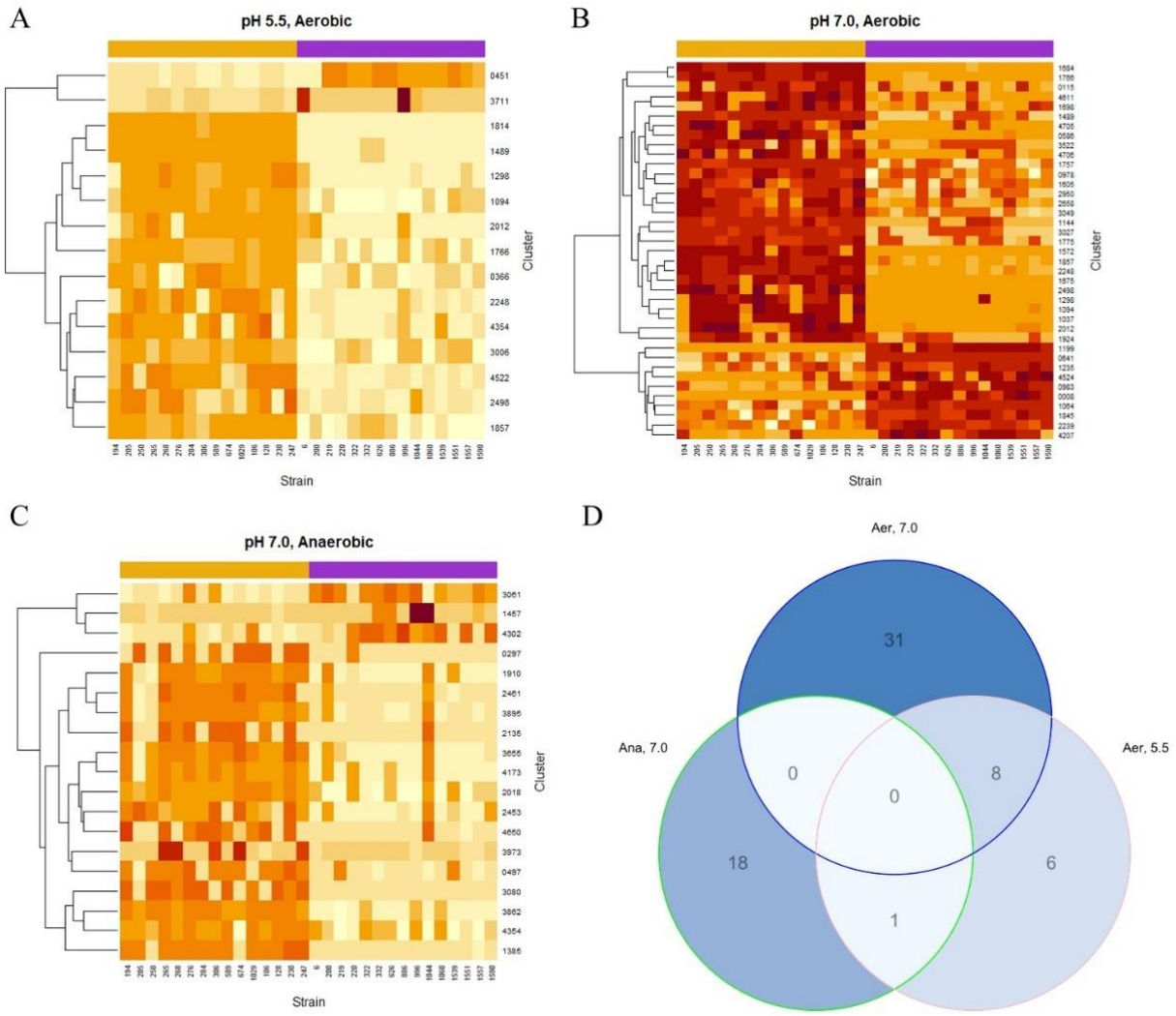


Figure 3.13 Continuation of Figures on Separation of Healthy and Atopic Eczema Strains

(A-C) Heatmap of significant features, scaled by feature. Significant clusters determined according to the volcano plots with limits $p < 0.05$ and folder change higher than 5-fold. Coloring according to intensity, high intensity (red), low intensity (yellow). Top most bar indicates whether the strains are either AE strains (burnt yellow) or HE strains (purple). Samples are seperated according to environment the strains were grown within with pH 5.5, aerobic (A); pH 7.0, aerobic (B); pH 7.0, anaerobic (C). (D) Venn diagram across the three environments for the significantly different clusters determined by the prior volcano plots. Shading according the number of clusters with darker shades indicating higher cluster number.

3.5 Development of Method to Capture Skin Metabolome and Applications

3.5.1 Development of Non-invasive Method (WET PREP) to Capture Stable Skin Metabolome

To validate that our method, WET PREP, which involves less sample prep and is easier to transition into clinical settings, the metabolome captured was compared to that of pre-wet swabs. The author previously published all figures from this section in the manuscript "Enhanced Access to the Health-Related Skin Metabolome by Fast, Reproducible and Non-Invasive WET PREP Sampling" (Afghani et al. 2021). Over 2500 metabolites were found on the skin, and the numbers of metabolites were comparable between the two sampling methods, except for the lipid fraction being captured best by swab (Figure 3.14). There is a high overlap in the metabolites captured by both methods and method-specific metabolite detection (Figure 3.14). According to PCA, the metabolome is highly influenced by the sampling method, and there is denser clustering of the samples for WET PREP when using the reverse phase column (Figure 3.14). When comparing the samples, a core metabolome was found across the samples (Figure 3.14). When considering the majority of samples (>40, with the sample total being 44), the core metabolome sampled is larger when sampled by WET PREP than by pre-wet swab regardless of the column used (Figure 3.14). Overall, this indicates that the sampling method can affect the metabolome detected from the skin.

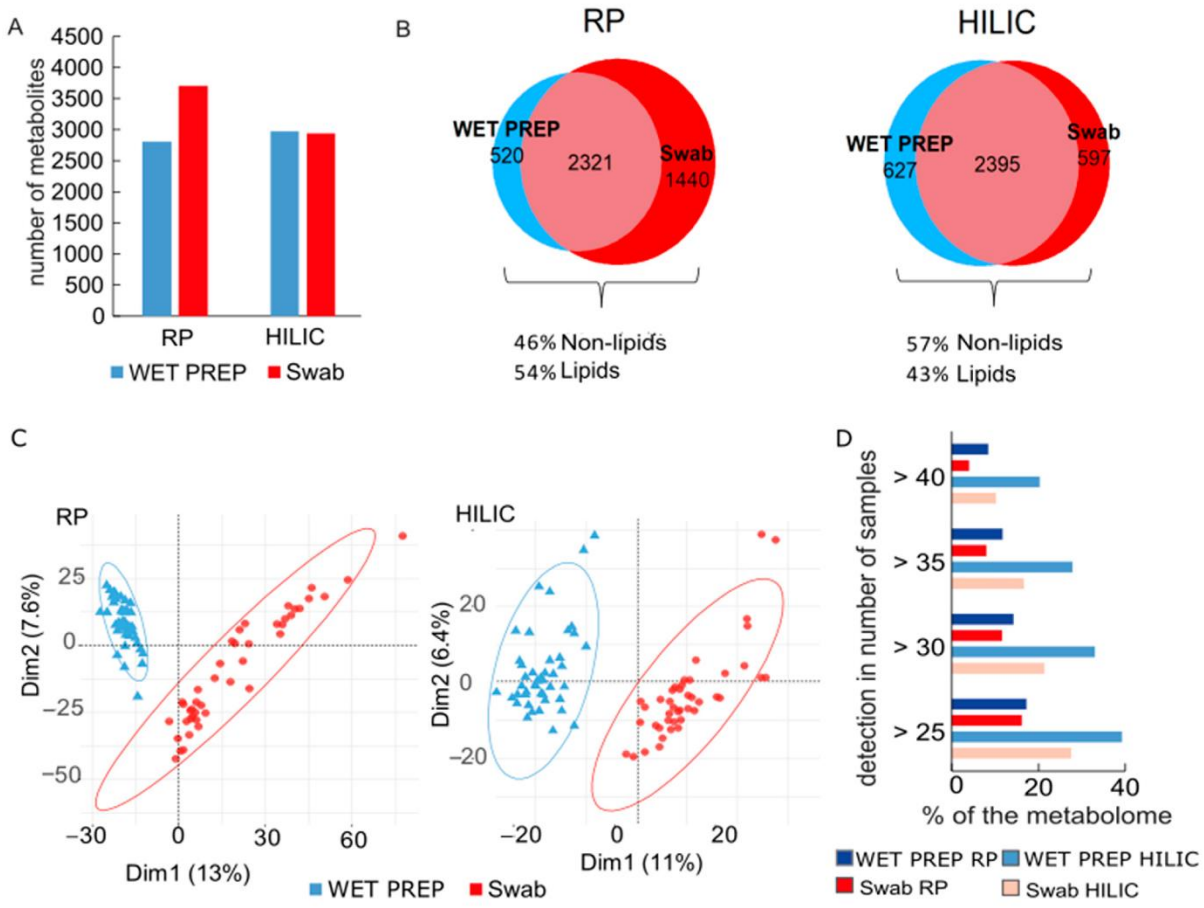


Figure 3.14 Comparison of Metabolome Detected from WET PREP and Pre-wet Swab

(A) Total number of annotated metabolites separated according to column used (RP or HILIC) and sampling method. (B) Venn diagrams of the total number of compounds according to sampling method with percentage of lipids-compounds that had to be present in at least 2 samples per sampling method to be considered. (C) Principal component analysis of the RP (left) and HILIC (right) metabolomes found in WET PREP (blue) and swab samples (red). (D) Percentage of shared metabolome across samples with HILIC (lighter colors) and RP (darker colors).

A dendrogram was made to determine if the city of residence and bilateral replicate grouping exists. This is because the samples were taken from two groups of individuals residing in two different cities, and current literature lacks information on whether bilateral replicates are similar for the metabolome. City of residence highly impacts the skin metabolome, with grouping according to the city of residence occurring after grouping based on the sampling method (Figure 3.15). In addition, when looking at the individuals, bilateral replicates grouped for all individuals sampled by WET PREP but not for pre-wet swab (Figure 3.15). The data confirms that WET PREP is more reliable regarding clinical replicability and suggests that the skin metabolome can change according to city of residence.

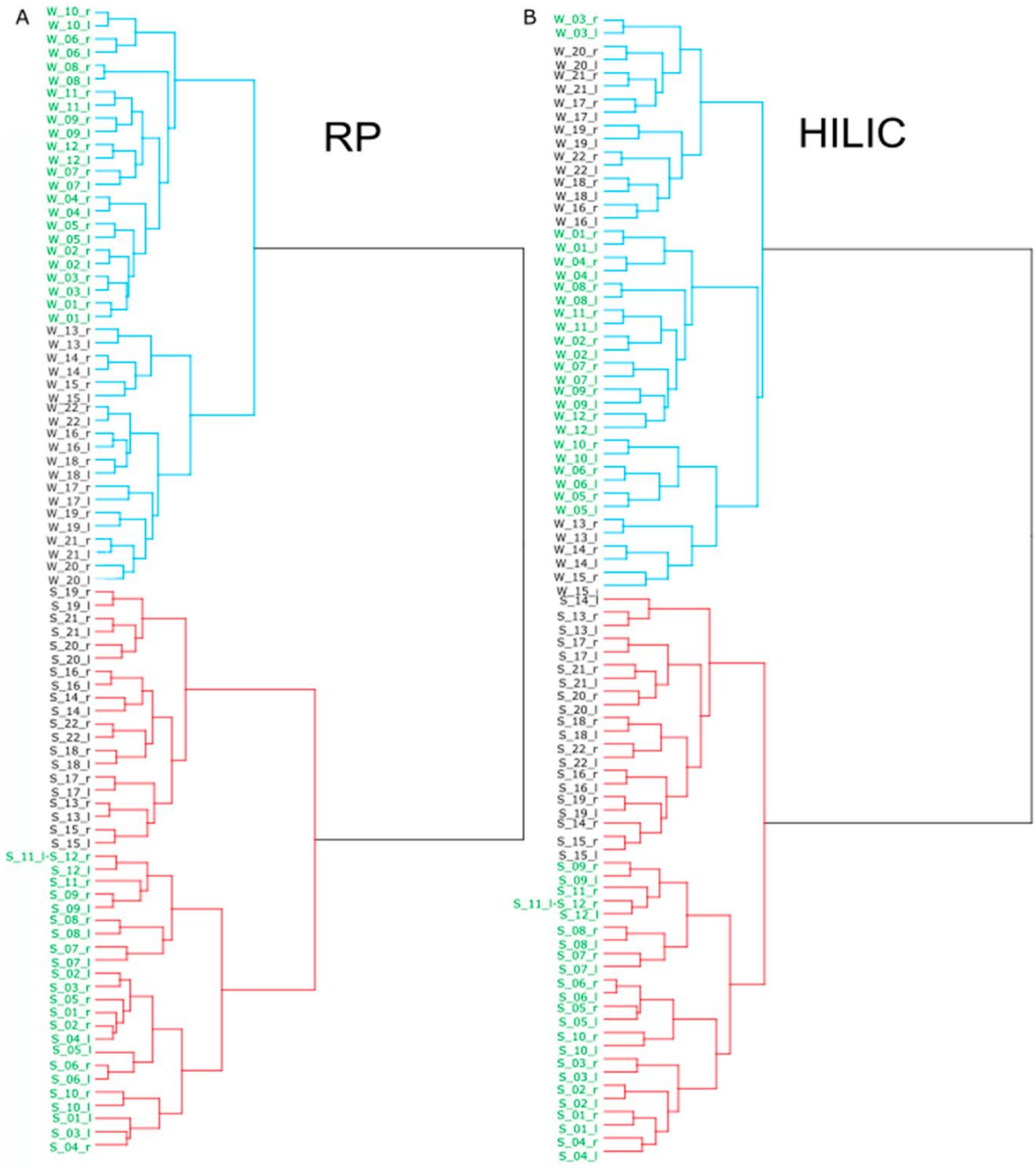


Figure 3.15 Dendrograms of Pre-wet swab and WET PREP

(A) RP and (B) HILIC. WET PREP samples noted in blue lines and swabs in red lines. Sample annotation is according to sampling method: WET PREP (W) and swab (S), individual (number following "W" or "S"), lateral side right (r) and left (l), and city of residence, location a (green) and location b (black).

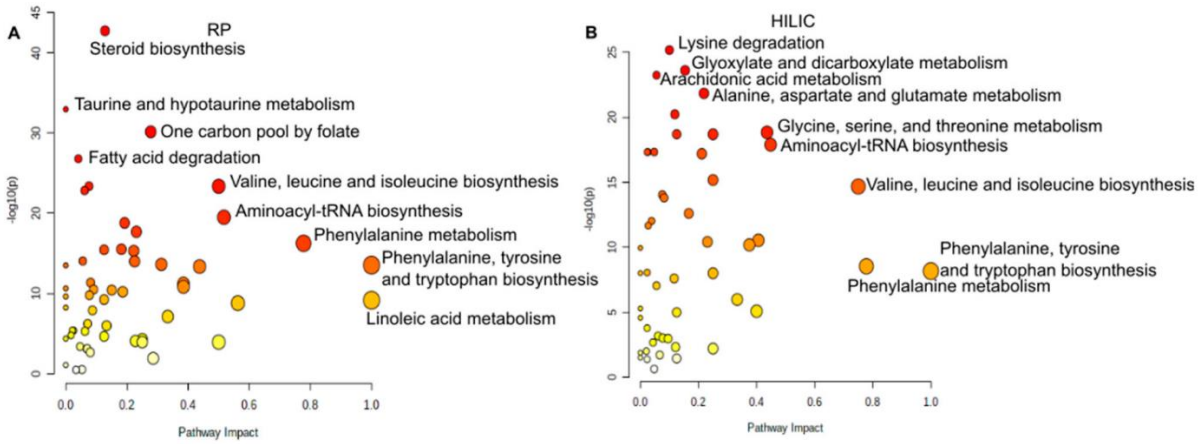


Figure 3.16 Pathway Analysis Chart of Significantly Different Metabolites Between WET PREP and Pre-wet swab

(A) RP and (B) HILIC. Metabolites that are significantly different between WET PREP and swab across each column type and with KEGG ID's, as determined by MassTRIX annotation, were run against the human KEGG database by MetaboAnalyst. The top 20 pathways are shown according to p-value and impact factor for out of degree centrality.

In addition to looking at the metabolome in general, the annotated metabolites found to be significantly different between the two sampling methods were cross-referenced to their usage in known metabolomic pathways as determined by the human KEGG database. From this, it is clear that there is differential detection of metabolic pathways depending on the sampling method used, for example, pathways involved in amino acid biosynthesis and catabolism (Figure 3.16). Also, select metabolite species previously published to be relevant within the skin were compared between the two sampling groups. The sampling method highly influences the detection of skin-relevant metabolites (Table 3.1). 63% of the amino acids and derivatives detected were detected more in WET PREP. For sugars, aromatics, nucleo(t/s)ides, and other acids: four out of eight metabolites were more highly detected by WET PREP (Table 3.1). This indicates that the sampling method does influence the detection of pathways and skin-relevant metabolites.

Table 3.1 Abundance of Metabolites of Skin Relevance with Regards to Sampling Method

Compounds of interest were compared for their detection in swabs and WET PREP samples. Exact m/z values (0.005 Da) and retention time were used for identification. All compound identification was verified with analytical standards. Log2 fold change WET PREP/swab shows the average intensities across all samples. (n.d.) denotes that the compound is not detected. Metabolites are grouped according to chemical type with their references, as seen on the far right. (*) denotes a reference from a serum study. Samples were tested for significance by Welch test: $p < 0.05$ (+), $p < 0.005$ (++), $p < 0.0005$ (+++). Scattered detection indicates partial detection in only some of the samples.

Category	Compound	Focus	RP		HILIC		Reference
			Significant Different Detection between WET PREP and Swab	log_2 Fold Change (Average WET PREP/Average Swab)	Significant Different Detection between WET PREP and Swab	log_2 Fold Change (Average WET PREP/Average Swab)	
Amino Acid	Taurine	Age	n.d.		+	0.04	Kuehne et al., 2017
	Serine	Psoriasis	+	0.21	+	0.21	Kim et al., 2009
	Proline	Age	+++	0.11	+	0.03	Kuehne et al., 2017
	Threonine	Age	n.d.		++	0.10	Kuehne et al., 2017
	Aspartic acid	Dock8 deficiency	n.d.		n.d.		Jacob et al., 2019 *
	Glutamine	Psoriasis	+++	1.19	+++	only WET PREP	Kim et al., 2009
	Glutamic acid	Psoriasis	+	0.61	−	−0.14	Dutkiewics et al., 2016
	Histidine	Cancer	+++	0.32	n.d.		Taylor et al., 2020
	Phenyl alanine	Psoriasis	+	−0.09	+++	−0.11	Dutkiewics et al., 2016
	Tyrosine	Age	+	0.06	+	−0.07	Kuehne et al., 2017
	Tryptophan	Age	+	−0.09	−	−0.04	Kuehne et al., 2017
Amino Acid Derivative	Hypotaurine	Dock8 deficiency	+++	only WET PREP	n.d.		Jacob et al., 2019 *
	Pyroglutamic acid	Skin	-	0.02	−	0.14	Joo et al., 2012
	Ornithine	Age	+++	0.72	scattered detection		Kuehne et al., 2017
Acid	Lactic acid	Psoriasis	−	−0.08	n.d.		Dutkiewics et al., 2016
	Retinoic acid	Age	+++	−0.22	n.d.		Kuehne et al., 2017
Sugar	Fucose	Age	n.d.		+++	−0.15	Kuehne et al., 2017

Category	Compound	Focus	RP		HILIC		Reference
			Significant Different Detection between WET PREP and Swab	log_2 Fold Change (Average WET PREP/Average Swab)	Significant Different Detection between WET PREP and Swab	log_2 Fold Change (Average WET PREP/Average Swab)	
	Glucose	Age	scattered detection		+	0.04	Kuehne et al., 2017
Nucleo(t/s)ides	Uracil	Age	−	0.03	+	−0.18	Kuehne et al., 2017
	Guanosine	Atopic Eczema	scattered detection		scattered detection		Jacob et al., 2019 *
Aromatic	Cresol	Age	scattered detection		+++	1.11	Kuehne et al., 2017
	Caffeine	Atopic Eczema	scattered detection		+	0.49	Jacob et al., 2019 *

3.5.2 Efficiency of WET PREP at Capturing Volatile Skin Metabolome

In addition to capturing the stable metabolites, we wanted to determine the applicability of WET PREP towards capturing the VOCs present within the skin; therefore, we compared our method to direct measurement of the skin with the SICRIT ionization source. There is a high overlap in the metabolites detected in both methods, and the abundance of the metabolites is not significantly different across the two methods, according to the volcano plot (Figure 3.17). In addition, to determine WET PREP's applicability for diagnosing AE, VOCs were collected by WET PREP. According to the PCA, there is distinct clustering between healthy and AE samples, but according to the dendrogram, healthy and AE NL samples are more similar (Figure 3.18). This preliminary study shows the potential of WET PREP as a tool to measure skin VOCs and diagnose AE.

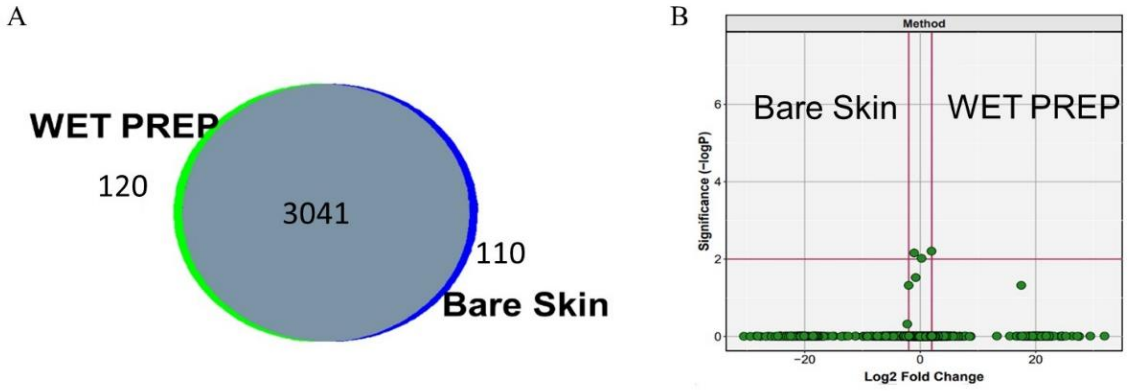


Figure 3.17 High VOCs Overlap Between WET PREP and Direct Skin Sampling

(A) Venn diagram of volatile organic compounds (VOCs) detected by WET PREP (green) and bare skin (blue) sampling when measured by the same ionization source. (B) Volcano plot across the two sampling methods with limits $p \leq 0.05$ and fold change higher than 5-fold.

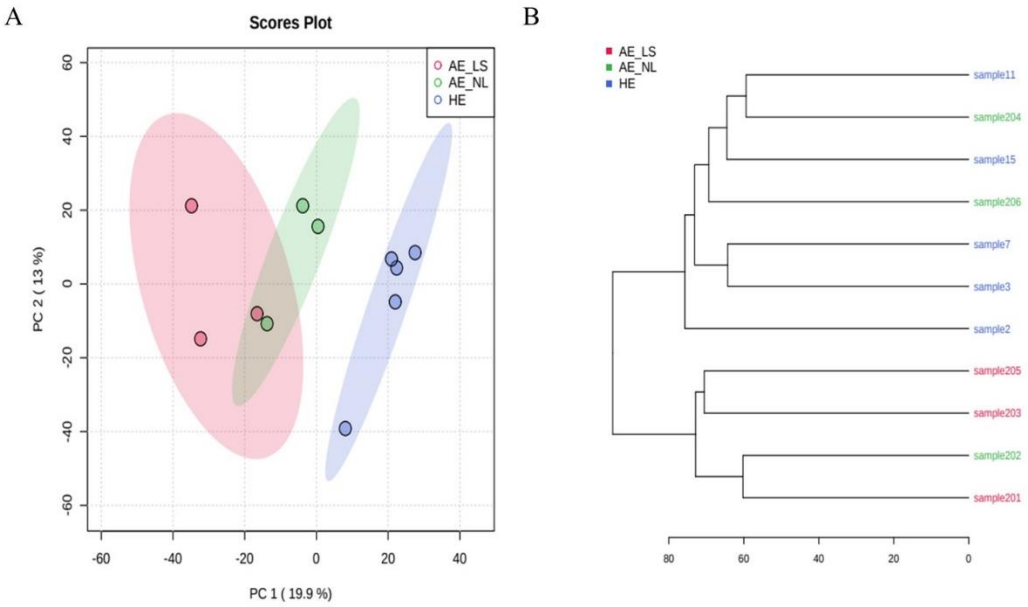


Figure 3.18 Discrimination of AE and HE Volatilome

(A) Principle Component Analysis of the volatile organic compounds (VOCs) detected by WET PREP on healthy (blue) and AE skin (LS, red; NL, green). (B) Dendrogram of the VOCs. Abbreviations: Atopic eczema (AE), lesional (LS), non-lesional (NL).

Chapter 4 - Discussion

AE is increasing in prevalence (H. Williams et al. 1999; Odhiambo et al. 2009; Bantz, Zhu, and Zheng 2014; T. Bieber et al. 2016) and is just a stepping stone on the route of atopic march (Bantz, Zhu, and Zheng 2014; Hill and Spergel 2018). This thesis aimed to explore the impacts of the micro-environment, specifically those suspected to play a role in AE pathogenesis, on skin homeostasis and to create a more clinically feasible metabolomic sampling method for skin health diagnosis. Because oxygen can be influential for many of the topics covered and has yet to be thoroughly covered in the context of AE, a large portion of this thesis focuses on examining the influence of oxygen. Bacterial load is higher within AE in general and within the context of Staphylococci and *S. aureus.* The oxygen gradient within the skin has the potential to support anaerobic bacteria within the skin, and two of the three individuals sampled had higher levels of anaerobic bacteria within the skin. In addition, the relative abundance of the anaerobic microbiome is lower in AE, with severe AE having the lowest abundance. The environment (oxygen and pH) can influence *S. aureus*'*s* growth and secretions. Within higher pH environments, there are unique secretion signatures for AE and HE strains as opposed to lower pH environments. Lastly, WET PREP is a clinically feasible metabolomic sampling method with coverage of metabolites highly overlapping with pre-wet swab sampling and has the potential for usage towards AE diagnosis. Overall, the results found in this thesis highlight the importance of the environment and sampling method in understanding and diagnosing skin health.

4.1 Bacterial Abundance in Relation to Skin Depth and Disease

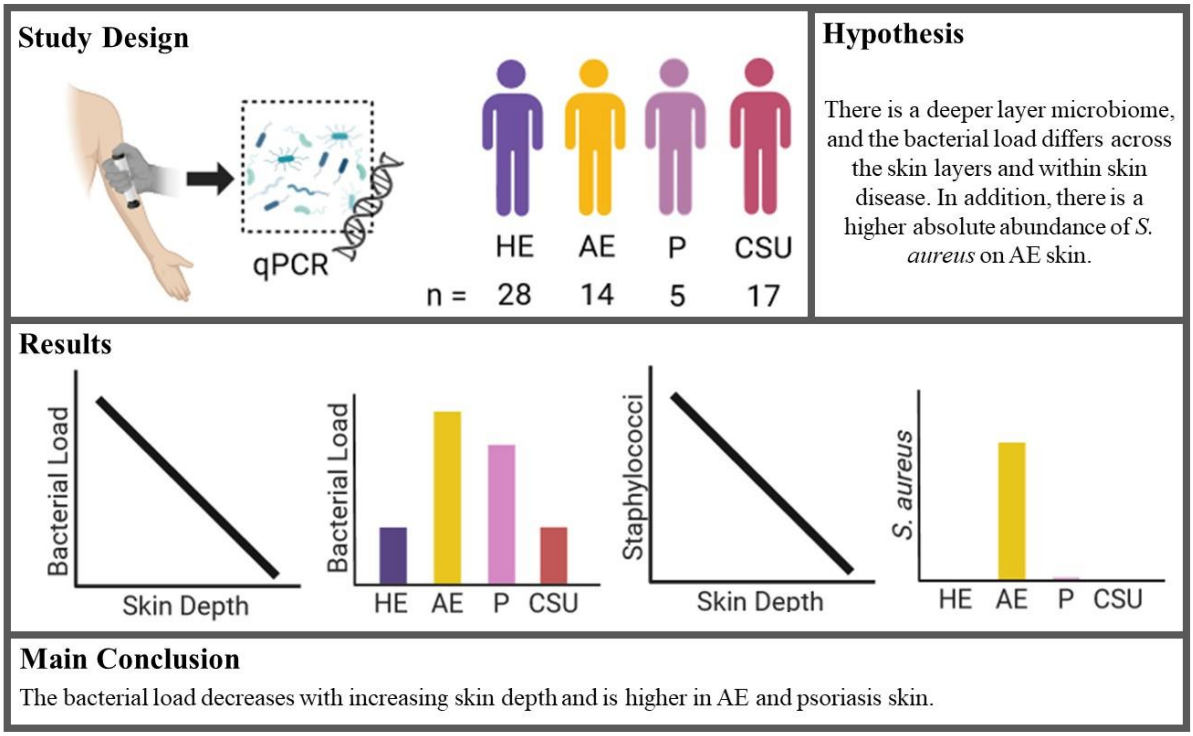


Figure 4.1 The Bacterial Load Decreases with Skin Depth and Increases with Skin Disease

Figure created with BioRender.com. Graphical summary of the section "Bacterial Abundance in Relation to Skin Depth and Disease."

Bacterial quantification is vital because it can help determine if a disease has a microbial signature. Absolute bacterial abundance is useful because certain infections can be bacterial dose-dependent (Wang et al. 2021), and cell density is critical for quorum sensing. To measure bacterial absolute abundance, i.e., bacterial load, qPCR was done. Measuring bacterial load by qPCR of the 16S ribosomal RNA gene is not completely new, with a similar study performed by (Quan et al. 2020). The combination of bacterial load and relative abundance data increases the power of the analysis. Quantifying bacterial load is helpful because it allows for quick quantification of total bacterial numbers without needing various, different culture plates because bacteria have different nutritional needs. According to relative abundance studies, AE is a microbial-influenced disease, with microbial dysbiosis in conjunction with *S. aureus* overgrowth (Hrestak et al. 2022). Still, as suggested by Reiger, Traidl-Hoffmann, and Neumann, relative abundance might not be a good enough biomarker (Reiger, Traidl-Hoffmann, and Neumann 2020). Recently, De Tomassi et al. found that the bacterial load was

higher in AE than in healthy (De Tomassi et al. 2023), but it is not yet known if this is universal across the skin depths.

The bacterial abundance was measured across the layers of the skin within AE (n = 14) with healthy (28), psoriasis (5), and CSU (17) samples taken as control groups. With regards to bacterial abundance, the results display that there is a differential abundance for both differing skin depths and skin diseases (Figure 3.1). The bacterial load was determined to decrease with increasing skin depth across all health statuses (Figure 3.1). Higher bacterial loads were found within the skin for AE and psoriasis but not for CSU (Supplement Figure 7.1). Alongside the bacterial load decreasing with depth, the abundance of Staphylococci also decreases with increasing depth (Figure 3.2). *S. aureus* was found predominantly among AE participants, and its location goes across the skin layers (Figure 3.2). This study confirms that the bacterial load is increased in AE and that this increase is not limited to the surface of the skin.

First, the capability to detect 16S was determined for each participant. The percentage of individuals with detectable 16S was not consistent across all skin types (AE, P, CSU, HE, NL, and LS), and psoriasis NL skin was the only group to have 100% detectable 16S in both the surface and within skin samples (Figure 3.1). This difference in detection could not be due to locational differences or hygiene habits because all patients were sampled at the same location and instructed not to bathe or use cremes. Despite the similarity in sampling location, the inner elbow is known to have lower levels of bacteria than other sampling locations (Gao et al. 2010). In addition, the sampling method does impact the bacterial biomass collected (Kong et al. 2017), and the samples taken here were tape strips. Tape stripping has been reported to provide larger amounts of biomass (Kong et al. 2017), but when directly compared to dry swabs, the quantity was reduced from 10^7 to 10^4 16S rRNA copies (Hülpüsch 2021). These results suggest that the presence of a skin microbiome is not universal for all individuals, but further studies with swabs should be performed to eliminate the potential sampling bias and validate the results in a larger cohort.

Of those that have detectable 16S copies, the number of copies decreases with increasing depth (Figure 3.1). This occurs regardless of disease status. The presence of this decrease is consistent with Zeeuwen et al., where in their study, bacteria reads were found to decrease from the surface of the skin to TS15, but contrary to Zeeuwen et al., 16S reads were found even deeper, past TS15, in the skin (P. L. Zeeuwen et al. 2012). This contradiction could be due to differences in sampling location, where the microbial composition is known to differ

according to the microenvironment (Costello et al. 2009; Grice et al. 2009), and the microenvironment's influence could also be true for the bacterial load. Zeeuwen et al. sampled the upper buttock (sebaceous), while this study was done on antecubital fossa (moist) (P. L. Zeeuwen et al. 2012). The presence of a microbiome was previously found as far down as the basal layer of the skin (Natsuga, Cipolat, and Watt 2016) and the dermis (Bay et al. 2020), and the higher level of bacteria in the epidermis as compared to the dermis is also known (Nakatsuji et al. 2013a). Also, based on the range of copies found within each tape strip level, there are inter-individual differences (Figure 3.1) that align with the relative abundance of the subcutaneous microbiome results of (Nakatsuji et al. 2013b) and the surface microbiome results of (Grice et al. 2009; Costello et al. 2009).

Bacterial load also differs according to disease status; in AE, the 16S copies are higher than in healthy (Supplement Figure 7.1). This bacterial load difference extends as far down as TS40 (Figure 3.1), which is right after the point at which the tape strips, for healthy individuals, have entirely removed the stratum corneum (Olesen et al. 2019; Sølberg et al. 2018) and for AE individuals, the tapes are still within the epidermis but past the stratum granulosum (Kim et al. 2019). After this point, the differences in bacterial load between healthy and AE are not as definitive. This lack of difference may be due to the tape strips for AE now reaching the dermis and being compared to epidermal levels of healthy bacterial load. The abundance of bacteria is higher in the epidermis than in the dermis (Nakatsuji et al. 2013a), which could lead to false comparisons.

For both AE and psoriasis, there is a higher bacterial load than healthy at TS2, which is only significant when compared to LS skin (Supplement Figure 7.1). Only at TS20 did both NL and LS skin become significantly different compared to healthy (Supplement Figure 7.1). For both AE and psoriasis, the results from the surface of the skin are consistent with the literature (Quan et al. 2020, 202; De Tomassi et al. 2023). Although psoriasis is a disease known for epidermal thickening (de Rie, Goedkoop, and Bos 2004) and skin scaling (Nikam et al. 2023), AE is not. Since this pattern occurs for both, it suggests this is not an epidermal thickening effect, and a follow-up study measuring the epidermal thickness and bacterial load across different depths is warranted for confirmation. Another possible reason could be that bacterial load increases only when a certain level of defects in the skin barrier occurs. The skin barrier is still intact in CSU, and the bacterial load is not increased in CSU (Supplement Figure 7.1). TEWL is one measure of skin barrier integrity, with lower TEWL corresponding to a more intact barrier. There is no difference in TEWL between CSU and healthy individuals

(Pham et al. 2017). Yet, in mice with a defective cornified layer, there is an increase in bacterial load (Natsuga, Cipolat, and Watt 2016), and TEWL is positively associated with bacterial load (Jinnestål et al. 2014). Therefore, the high bacterial load can only be seen at the skin's surface when there is an extreme disruption to the physical barrier, like lesions. This does not explain why at TS20, the bacterial load of LS and NL samples is significantly higher than healthy samples (Supplement Figure 7.1). A possible explanation is that the surface of LS skin is simply a reflection of the bacterial load from within the epidermis of NL skin. Zeeuwen et al. found that after injury, the composition of the surface microbiome is more reflective of the deeper layer microbiome, which could also be true for bacterial total abundance (P. L. Zeeuwen et al. 2012). In addition, the differences between LS and NL skin could be due to other extraneous factors. Compositionally the skin microbiome community is temporally stable, but abiotic factors can influence it, and one cannot simply control for every environmental factor (Pistone et al. 2021). Lastly, the difference in quantities for LS and NL skin at TS2 could be because sampling captures only a snapshot and perhaps because NL diseased skin starts to shift the environment to improve transient bacteria colonization at the surface, but not enough for full dysbiosis. With regard to microbial diversity, NL psoriasis has been suggested to be the transition point to LS psoriasis (Quan et al. 2020, 202). Ultimately this warrants further study where temporal qPCR measurements alongside compositional measurements can be compared to determine if the higher bacterial load at the surface of NL and LS skin is due to transient bacteria colonization and whether the levels of transient bacteria are higher in LS skin than NL. Overall, there are many potential reasons for the higher bacterial load in AE skin and potential explanations as to why the significance of this increase for both NL and LS skin is only seen at TS20.

Since there is an increase in bacterial load for AE, the immune system might secrete more antimicrobial peptides (AMP). Although AE and psoriasis have higher bacterial loads (Supplement Figure 7.1), they differ in AMP levels. AMP are high in psoriasis but low in AE, possibly due to Th2-derived cytokines found in AE that inhibit AMP expression (Godlewska et al. 2020). AMP expression is not uniform in AE, which could be due to stratification in cytokine expression. Within the acute disease, there is Th2/Th22-based inflammation, and within the chronic disease, there is Th1/Th17 (Hrestak et al. 2022). As of yet, there has not been a study correlating markers of inflammation with AE bacterial load, and such studies could confirm if the bacterial load, in general, is a driver of AE inflammation. Previous studies have been performed connecting inflammation markers with bacterial composition. Changes

to the microbiome composition in AE are multifactorial, and one such factor is changing AMP expression (Nakatsuji et al. 2023). In a mice study, Th2 inflammation reduces *S. aureus* antagonistic CoN Staphylococci proliferation, providing a unique opportunity for *S. aureus* proliferation. Then under Th17 conditions, AMP production rises, inhibiting growth for *S. aureus* and CoN Staphylococci (Nakatsuji et al. 2023). The total abundance of Staphylococci is particularly interesting for AE because AE is associated with Staphylococci (Aggarwal et al. 2022; Godlewska et al. 2020).

Species in the Staphylococci genus have been positively and negatively associated with disease, and even within each species, it's uncertain whether a species can be considered entirely commensal or pathogenic. Within AE, *S. epidermidis* can induce inflammation (Ochlich et al. 2023) and attenuates inflammation (M. M. Brown and Horswill 2020). Nevertheless, the larger grouping can provide initial information on the broader changes within the disease. Staphylococci and *S. aureus* were quantified by qPCR to determine depth-associated and disease-associated effects. Although Staphylococci and *S. aureus* have been previously found to be associated with AE, this is the first study looking at their total abundance in relation to skin depth.

The quantity of Staphylococci cells generally decreases with increasing depth (Figure 3.2). Similar results were found in (C. J. Barnes et al. 2022) and align with the findings of (Nakatsuji et al. 2013a), where the phylum firmicutes and the class bacilli were found within each individual but inconsistently across the sections of the skin (epidermis and dermis). Staphylococci are present on the surface of healthy individuals' skin (Figure 3.2), and this supports prior literature where Staphylococci are considered skin residents, especially at the moist skin site of the antecubital fossa (Hrestak et al. 2022). The presence of Staphylococci within healthy individuals is very low within the epidermis (Figure 3.2). Despite this, Matard et al. could culture CoN Staphylococci from the sub-epidermis (Matard et al. 2020). Staphylococci can survive intracellularly (Onyango and Alreshidi 2018), and one possible reason for the low Staphylococci counts could be the intracellular penetration of keratinocytes within the epidermis. This penetration could result in Staphylococci DNA not being released during the bead-beating step of DNA extraction because both the keratocyte cell wall and the bacterial membrane must be disrupted. Further tests with harsher bead-beating must be performed to confirm. For healthy individuals, the quantity of Staphylococci cells at the skin's surface strongly correlates to skin pH, where more Staphylococci are found at a more acidic pH (Figure 3.3). Select species of Staphylococci tend to prefer more acidic conditions (Lambers

et al. 2006), and a follow-up compositional study would be necessary to determine which select species are driving this pH correlation. Staphylococci can be found in healthy individuals, and the cell number decreases with increasing depth across all health statuses.

At the surface and within the epidermis, there is a significantly higher quantity of Staphylococci cells in AE LS skin relative to healthy (Supplement Figure 7.2). The pattern of higher levels of Staphylococci cells within the LS samples is not only in AE but also in psoriasis samples (Supplement Figure 7.2), and this suggests the biological reasoning extends beyond the immune system since the immune response differs between these two diseases (Godlewska et al. 2020). The differentiation in response still results in the same observation; Staphylococci are overgrown in LS skin. In addition, CSU, which has a more intact barrier, does not have differences in Staphylococci abundance as compared to healthy (Supplement Figure 7.2). The genus of Staphylococci are facultative anaerobic, so they could live in the deeper regions of the skin and then overgrow once the skin is scratched. Staphylococci can colonize deeper by traveling down the hair follicle (Bay et al. 2020) and, as stated before, by swimming through the keratinocytes like an intracellular pathogen (Al Kindi et al. 2019). Staphylococci are found in the deeper layers of NL AE and psoriasis skin (Figure 3.2). Perhaps, Staphylococci are hidden deep within the NL skin and adapted to overgrow with a flux of oxygen induced by scratching. This concept will be discussed in a later section of the thesis.

The quantities of Staphylococci cells increase from healthy to AE NL to AE LS (Supplement Figure 7.2). The observation of higher Staphylococci counts in AE NL skin aligns with the previous literature, where at the surface and the TS5, there are higher relative levels in AE NL skin relative to healthy (C. J. Barnes et al. 2022). AE NL skin has a more acidic pH than its LS counterpart (Zainal et al. 2020), and more alkaline pH improves bacteria-host cell adhesion (Miajlovic et al. 2010), so the rise in Staphylococci could be a result of pH change. In addition, the skin hydration at the cubital fossa is lower for LS versus NL skin (Zainal et al. 2020), and skin hydration also improves Staphylococci adhesion (Feuillie et al. 2018). The quantity of Staphylococci negatively correlates to severity within AE LS samples, but only for local EASI and not for SCORAD (Figure 3.3). Both measurements take into account symptoms of AE, redness, swelling, etc. (Byers et al. 2018; Hanifin et al. 2022), but local EASI does not take into account the total surface area affected and therefore, might be a more accurate measurement for localized change like the microbiome. The lack of correlation to SCORAD contradicts Zeng et al., where the relative percentage of Staphylococci correlates to SCORAD (Zeng et al. 2020), but this difference in result could be due to comparing different metrics

absolute versus relative abundance to SCORAD. Overall, Staphylococci cells are increased in AE LS skin, with correlations warranting further study to elaborate if this is a select species-driven effect.

As stated before, by detecting the genus as a whole, both the pathogenic and immune stimulatory *S. aureus* (K. Matsui and Nishikawa 2005) and the Staphylococci, which have been shown to compete against the pathogenic *S. aureus* and alleviate inflammation, are measured (Traisaeng et al. 2019; J.-J. Yang et al. 2018). Because *S. aureus* is overgrown, associated with AE, and can predict AE severity (Hülpüsch et al. 2020 a), the quantity of *S. aureus* was also measured. *S. aureus* was only found in AE skin and is present down to TS40 for AE LS samples (Figure 3.2). Interestingly, past TS40, where Staphylococci are detected, very few samples have quantifiable levels of *S. aureus*, hinting that deeper in the skin, there is a shift in the abundances between the Staphylococci species (Figure 3.2). In nasal studies, *S. aureus* is found in the deeper layers of the epithelium (Hanssen et al. 2017), and according to Nakatsuji et al., *S. aureus* can penetrate the dermis (Nakatsuji et al. 2016). There is a higher *S. aureus* bacteria load in LS skin than NL (Figure 3.2), which is supported by the literature (Nakatsuji et al. 2017; van Mierlo et al. 2022; De Tomassi et al. 2023). The quantities of *S. aureus* among AE NL samples do not account for all the Staphylococci detected (Figure 3.2). Further studies quantifying other species of Staphylococci within these samples would be beneficial to confirm the observation that the species ratios change across the layers of the skin. The observation that *S. aureus* was detected in higher quantities at the skin's surface for LS samples is also the first hint that perhaps the environment of the lesions positively encourages *S. aureus* growth, which will be explored later in this thesis.

Overall, regarding absolute abundance, there are more bacteria, Staphylococci, and *S. aureus* in AE, with several potential directions for future study. Despite these results, several limitations in this study must be considered. As highlighted earlier, tape stripping collects lower biomass levels (Hülpüsch 2021), which could affect the bacterial kingdom, genus-specific, and species-specific results. However, tape strips are beneficial because they allow for deeper layer sampling; combining tape stripping and swabs would be more recommended for future studies. Another limitation, specifically for the total bacteria abundance results, is that 16S rRNA copies do not directly correspond to bacterial cell numbers. The 16S rRNA gene can have several copies within one bacterial cell, and the number of copies depends on the taxa (Větrovský and Baldrian 2013); this distorts the true bacterial load. Lastly, as a final point to keep in mind, the measurement of DNA does not necessarily mean that the bacteria is viable

(Emerson et al. 2017), and these studies should always be supported with in vivo or culturing studies. Nevertheless, the results from this study show that skin depth and bacterial load are important factors in the pathogenesis of AE.

4.2 The Anaerobic Microbiome is Present in the Skin and Reduced in AE

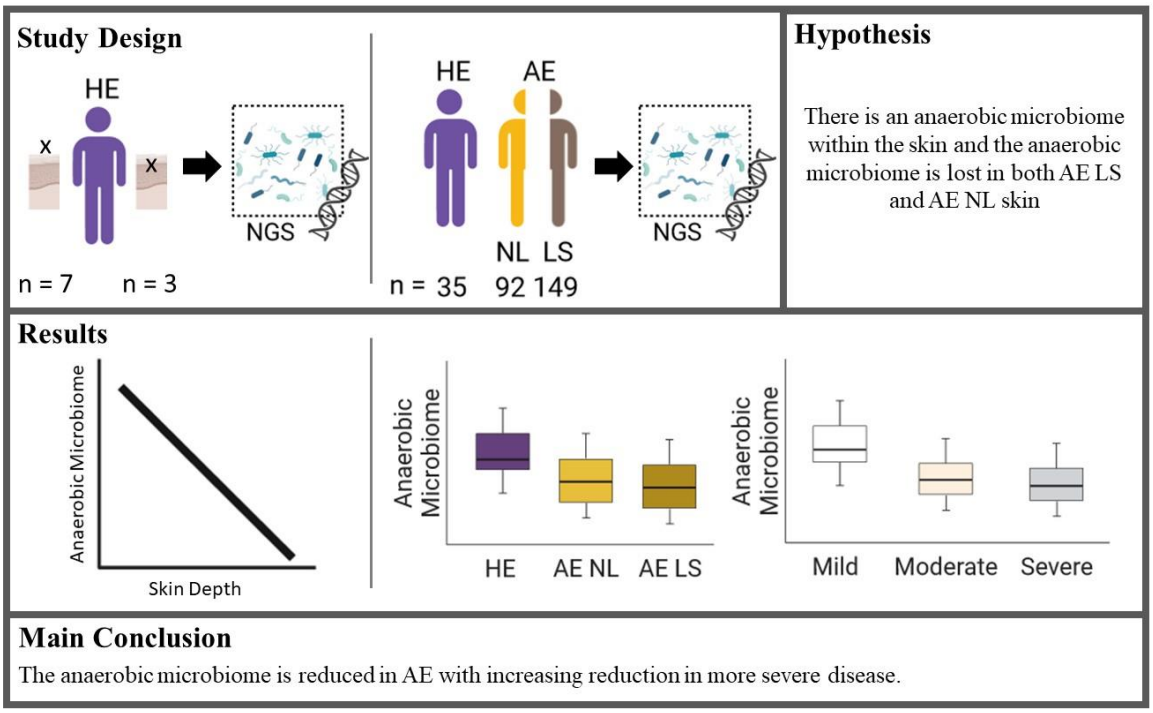


Figure 4.2 The Anaerobic Microbiome is Reduced in AE Skin

Figure created with BioRender.com. Graphical summary of the section "The Anaerobic Microbiome is Present in the Skin and Reduced in AE."

In addition to grouping bacteria according to genus, as seen in our previous section, the microbiome can also be grouped based on various inherent factors, and grouping the bacteria according to oxygen tolerance is important to further understanding AE pathogenesis. Anaerobic and facultative anaerobic bacteria can produce immune-modulating factors like SCFAs, which have been shown to influence the skin's health (Schwarz, Bruhs, and Schwarz 2017; Traisaeng et al. 2019). In addition, anaerobic bacteria can influence keratinocyte AMP production (van der Krieken et al. 2023). In FLG mutated AE skin, gram-positive anaerobic cocci are in lower relative abundance (P. L. J. M. Zeeuwen et al. 2017). FLG is a skin protein that connects keratinocytes, strengthening the physical barrier (Simpson, Patel, and Green 2011; Furue 2020). As a potential subsequent consequence, FLG may also help maintain the oxygen tension within the skin. Naturally, a mutation in such a gene would disadvantage anaerobic bacteria, which cannot survive in the presence of oxygen.

Chapter 4 -The Anaerobic Microbiome is Present in the Skin and Reduced in AE

Anaerobic bacteria, such as *Fingoldia magna* (van der Krieken et al. 2023; Murphy and Frick 2013) and the Anaerococcus genus (Oh et al. 2013), have been commonly isolated or detected on the skin. In addition, across the skin, the DNA of anaerobic bacteria is detected in the epidermis and the dermis, with 65% of the species found only in the epidermis being anaerobic (Bay et al. 2020). Reasons for anaerobic colonization are still under consideration. Bay et al. suggest that the bacteria may live within the anoxic hair follicles (Bay et al. 2020), but they could also take advantage of the oxygen gradient within the skin. Next-generation sequencing was performed to confirm the detection of anaerobic bacteria within the epidermis and look into its role in AE. Next-generation sequencing, i.e., 16S microbiome sequencing, can be used to determine the microbial composition of a matrix, and the cross-reference of the bacteria detected to the literature can also help with understanding the relative abundance of anaerobic bacteria.

The anaerobic bacteria across skin depths (surface, n = 7 and deeper layers, 3) and in AE (HE, 35; AE NL, 92; AE LS, 149) were explored. The size of the anaerobic microbiome is highly individual-specific (Figure 3.4), but the relative abundance tends to increase within the skin (Figure 3.4). The anaerobic microbiome is lost in AE, which occurs in both lesional and non-lesional skin (Figure 3.5). In addition, with more severe AE, the relative abundance of anaerobic bacteria decreases (Figure 3.5). No select species drives this decrease in AE, highlighting that the anaerobic microbiome altogether is important (Figure 3.5). These studies show that the anaerobic microbiome is present within the skin and showcases its importance in AE.

Whether the samples came from the surface or within the epidermis, the relative number of anaerobic bacteria was individual-specific (Figure 3.4). This supports the previously reported inter-individual variability of skin microbiome research (Costello et al. 2009; Grice et al. 2009). The inter-individual variability in anaerobic relative abundance at the skin's surface was also confirmed in our larger ProRaD study (Supplement Figure 7.4). This study shows that the relative abundance of anaerobic bacteria is independent of prior bathing, sex, and age (Supplement Figure 7.6, Supplement Figure 7.7). Despite this, as more thoroughly described in section 1.1.4, there are a variety of other factors (pollution, physical activity, etc.) that can impact skin microbiome composition, and not all factors could be accounted for in these studies. Within healthy individuals, there is a general trend of higher anaerobic bacteria frequency in the deep layers of the skin (Figure 3.4). This study was preliminary and limited, though, by the low participant numbers and the undefined skin section of the deeper skin

samples. Because of this, a follow-up study with larger participant numbers and a more clarified location on the epidermal layer must be performed to be certain of the increase in relative abundance and where it's located. In addition, from the taxonomic table, 23% of OTUs detected could not be labeled due to contradictions in the literature or a general lack of published information and testing regarding oxygen tolerance (Figure 3.4). This highlights the need for further or re-testing of species to provide essential information for broader stratification and testing within microbiome studies. Despite the various limitations of the study, it does confirm that anaerobic bacteria can be found within the skin.

The anaerobic microbiome is changed in AE (Figure 3.5). AE has been defined as a disease with observable microbiome dysbiosis (Rie Dybboe Bjerre et al. 2021; Demessant-Flavigny et al. 2023), itchy skin (Roth and Kierland 1964; Bhattacharya, Strom, and Lio 2016), and an impaired skin barrier (Sugiura et al. 2005; Palmer et al. 2006; K. C. Barnes 2010; Agrawal and Woodfolk 2014; Moosbrugger-Martinz et al. 2022; Katsarou et al. 2023). This leads to the idea that the anaerobic microbiome is reduced in AE. Fyhrquist et al. showed a reduction in the anaerobic microbiome between healthy and AE skin. Unfortunately, their study only included moderate-to-severe AE and did not explore further NL and LS differences and the relationship to severity (Fyhrquist et al. 2019). Although they used a different sampling method, phosphate-buffered saline wash, our results confirm this difference (Figure 3.5).

The relative frequency of anaerobic bacteria decreases from healthy to AE NL to AE LS (Figure 3.5), which aligns with the concept that the skin is more perforated in the same order. Confounding factors that can be involved in the abundance of anaerobic bacteria are only seen for NL skin (Supplement Figure 7.8). Based on multiple regression, these confounders are skin microenvironment and treatment, i.e., topical emollients. These confounders can influence if the relative abundance of the anaerobic microbiome can differentiate between HE and AE NL skin (Supplement Figure 7.8). The influence of treatment may be because the cremes, depending on their ingredients, can influence tight junctions and FLG expression. For example, *Spirodela polyrhiza* extract and *Olea europaea* leaf extract can upregulate the expression of skin barrier proteins (Katsarou et al. 2023). Also, high glucose application can upregulate FLG expression (Katsarou et al. 2023), leading to a stronger skin barrier with lower oxygen penetration. Steroids can negatively impact tight junctions, so further studies stratifying the treatment groups and anaerobic frequency would greatly broaden the understanding of why it is a confounding factor (Katsarou et al. 2023). In addition, it could

lead to more targeted treatments where the focus is improving anaerobic abundance within the skin.

The frequency of the anaerobic microbiome plays a role in severity (Figure 3.5). SCORAD has a weak yet significant negative correlation to the frequency of bacteria (Figure 3.5), indicating that the anaerobic microbiome is just a small part of the greater whole that influences AE severity. In addition, those with severe AE have significantly lower anaerobic frequency compared to mild severity cases (Figure 3.5), so the continual loss of the anaerobic microbiome might reflect the disease's progression. Since next-generation sequencing is performed and not qPCR, all measurements are relative to the bacteria content as a whole. In other words, these measurements regard relative abundance. Differences between the relative and actual abundance within the skin could occur, despite the findings of (Hülpüsch 2021), where *S. aureus* relative abundance highly correlated to qPCR cell number. Facultative anaerobic bacteria seem to take the space where the anaerobic microbiome is lost in AE (Figure 3.5). Therefore, the anaerobic microbiome correlation and pattern may be due to a rise in *S. aureus* relative abundance, and further stratification accounting for *S. aureus* abundance would be recommended. Despite this, further longitudinal studies on whether anaerobic abundance can predict disease severity is a future direction for this work. In addition, the observation of the anaerobic microbiome being potentially replaced by facultative anaerobic bacteria brings further questions to mind. Is this simply due to a flux in oxygen, because of deficiency in FLG (Moosbrugger-Martinz et al. 2022) and tight junctions (Katsarou et al. 2023) within AE, or is it more connected to scratching the itchy skin or both? It would be worth further study to determine the levels of the structural proteins and an individual's reported itchiness level in combination with measurements of the facultative anaerobic bacterial abundance. Overall, since the 16S rRNA gene is used to measure the relative frequencies of the bacteria, the limitations that 16S rRNA copies don't reflect the true abundance nor viability of bacteria put forth in section 4.1 still hold.

Also, the differences between healthy and AE skin are not specific to select anaerobic species and are an effect of the abundance of the anaerobic microbiome altogether (Figure 3.5). Rauer et al. found that SCORAD severity was best explained by a reduction in evenness for the AE microbiome and not richness, where select taxa are missing (Rauer et al. 2023). This means that the imbalance of the microbiome contributes to severity, and this concept supports the findings found here, where the anaerobic microbiome frequency is decreased in AE, throwing off the balance of bacteria within the skin and potentially influencing the severity of

the disease. Unfortunately, the ProRaD study was limited by only taking surface swab samples, and further validation in the more hypoxic deeper skin should be performed to confirm. Ultimately, whether the anaerobic microbiome drives AE pathogenesis or AE drives the changes in this biome is still a question that should be answered in further studies.

4.3 The Environment Impacts the Growth of *S. aureus*

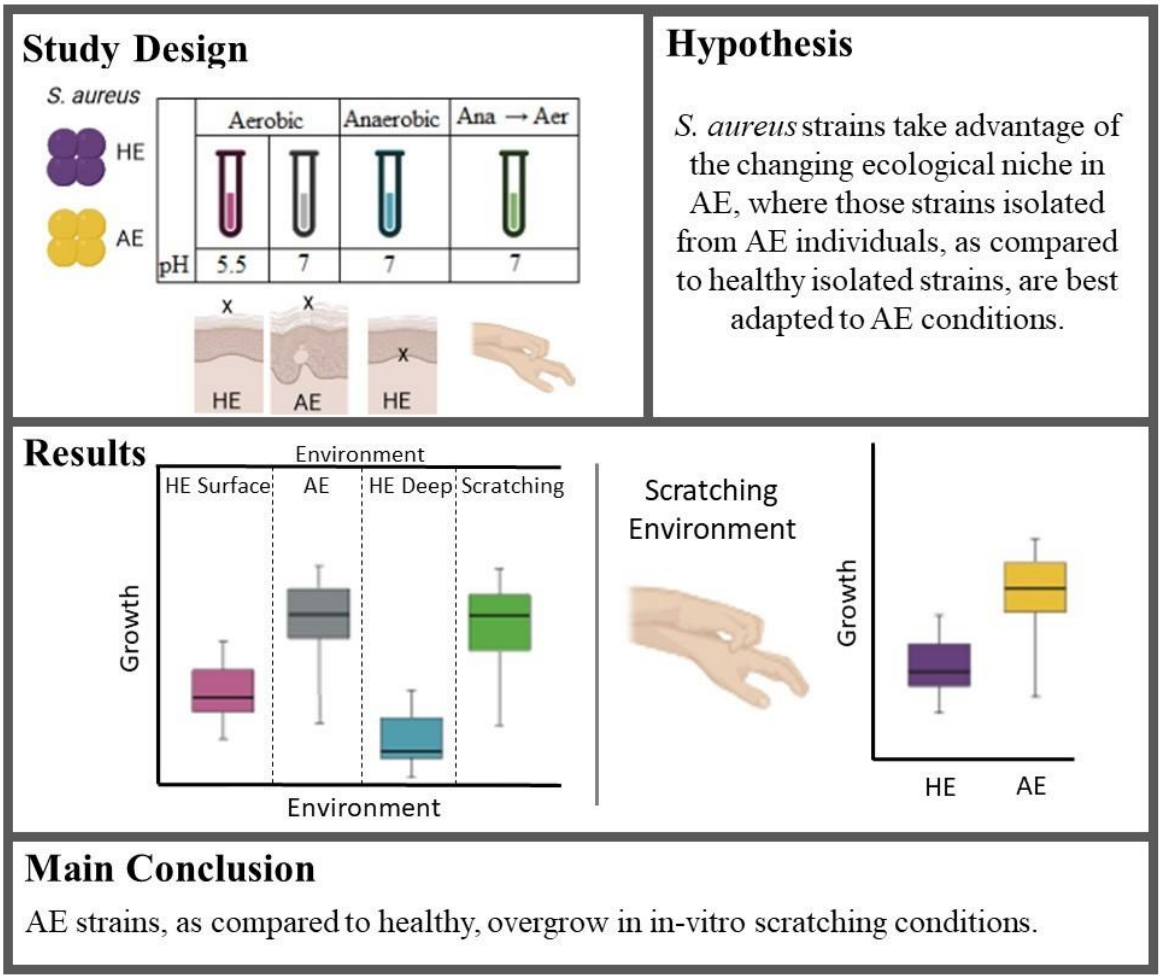


Figure 4.3 Scratching Leads to Overgrowth of AE *S. aureus* strains

Figure created with BioRender.com. Graphical summary of the section "The Environment Impacts the Growth of *S. aureus*."

Although the microbiome can be grouped in various ways to help explain the pathogenesis of AE from a microbial aspect, there is individual variability in the anaerobic microbiome's relative abundance and *S. aureus's* presence (Supplement Figure 7.5, Figure 3.2). AE has various endotypes (Czarnowicki et al. 2019), described in Table 1.1. Skin inflammation and itch is uniformly present in AE. However, the mechanisms behind the development of inflammation and itch and the pathways involved in each varies according to endotype. The skin barrier is weaker in diseased skin when compared to healthy skin; however,

the pathways by which this weakness develops are likewise unique to each endotype (Henderson et al. 2008; Kim and Leung 2018; Simpson et al. 2018; Czarnowicki et al. 2019; Chovatiya and Silverberg 2022). From this, it can be surmised that AE is just a category of diseases with different pathogeneses despite an outward similarity. The most prevalent factor in AE is skin itchiness (Chovatiya and Silverberg 2022), and combined with the knowledge that regions of the skin are anoxic and the effects of scratching change the skin's oxygen gradient, it is possible that this may play a role in the pathogenesis of one of AE's endotypes, *S. aureus* presence. In addition, oxygen is not the only factor that could influence the *S. aureus* endotype. Oxygen and pH within the skin are interconnected. In wound healing, oxygen tension and pH are correlated (Haller et al. 2021). This interconnection is also seen in irradiated skin, where both the pH and oxygen saturation of the skin are higher in irradiated skin (Auerswald et al. 2019). pH can influence the skin from *S. aureus* growth (Skowron et al. 2021; Proksch 2018b) to stratum corneum barrier formation (Proksch 2018b). pH also has a negative correlation to surface bacterial load. This is supported by (Lambers et al. 2006), where more acidic pH assists in the adhesion of bacterial flora to the skin. The skin pH is higher in AE skin (Panther and Jacob 2015), and according to the Deep Layer study, the abundance of Staphylococci was correlated to pH within healthy (Figure 3.6). Overall, this means that both oxygen and pH should be considered when testing the effect of the environment on one of AE's endotypes, colonization with *S. aureus*.

This section tested the effect of pH and oxygen on healthy and AE strains' growth. The anoxic environment has the largest impact on *S. aureus's* growth regardless of health status (Figure 3.7, Supplement Figure 7.9). Only among the oxygen flux environment, i.e., scratching condition, could a significant difference be seen in the growth of healthy and AE strains (Figure 3.7). Within this condition, AE strains had a spike in growth (Figure 3.7). This could explain the observation that *S. aureus* is overgrowth on the skin of patients with AE. Overall, this study highlights the importance of treating itch and preventing scratching in AE.

The potential growth of each *S. aureus* strain was highly influenced by the growth environment, where the largest impact was seen when the strains were grown in an anoxic environment (Figure 3.7, Supplement Figure 7.9). This is consistent with (Ledala et al. 2014), where oxygen limitation more greatly impacted *S. aureus* growth when compared to iron-limited conditions. There is stratification across the strains when grown in anaerobic environments, where skin strains grow better than nose strains (Supplement Figure 7.9). This is the first hint towards strain-level stratification regarding environmental response. As

reviewed in section 1.2.5 and recapitulated here, healthy and AE *S. aureus* strains trigger different inflammation responses. In addition, AE *S. aureus* strains are better, as compared to healthy nose strains, at adhering to the stratum corneum and this is independent of the clonal type of the strain (Fleury et al. 2017) in addition the pathways for adherence differ between HE and AE strains (Iwamoto et al. 2019). This AE and healthy strain grouping also extends to growth capabilities in response to environmental stimulation. Neither response to anoxic nor pH-reduced conditions differed between healthy and AE strains after checking for isolation location as a confounding factor (Figure 3.7). As stated before in sections 1.2.4, 4.1, pH influences *S. aureus*, and this thesis' research is limited because it was done in vitro and, therefore, cannot perfectly mimic native environments. Contrary to native environments where the skin is resistant to pH changes (Hülpüsch et al. 2020a), the growth medium was not buffered. It is not fully understood whether lower pH solely inhibits *S. aureus* growth or if this is simply an effect of specific acids present within the medium (Zhou and Fey 2020; Rode et al. 2010). According to (Rode et al. 2010), at pH 4.5, HCl is less effective at inhibiting growth than lactic acid and acetic acid. Therefore, further studies should be done with buffered media where different buffers are compared to determine if the pH effect on *S. aureus* growth is an overall effect and not a buffer-specific effect.

As stated earlier, itchiness is a uniform symptom in AE. A reason why healthy individuals can have *S. aureus* yet not develop AE is that there is a strain-specific *S. aureus* response to scratching conditions, i.e., oxygen flux. There is a significant difference between the growth of HE and AE strains in our simulated oxygen flux condition independent of isolation location (Figure 3.7). Although the lack of healthy skin strains limits this study, there is a linkage between nasal and skin carriage (Sakr et al. 2018), and there are identical clonal complexes between the nose and skin for AE individuals (Clausen et al. 2017). Also, Zhao et al. found aerobic growth between nose and skin isolates to be quite similar (Zhao et al. 2021). AE strains grew better than the optimal condition (aerobic, pH 7.0) (Figure 3.7), suggesting that *S. aureus* overgrowth is a consequence of scratching and *S. aureus* abundance is correlated to skin itchiness (Leung et al. 2009). In addition, scratching of the skin could assist in the deeper colonization of *S. aureus*, and the severity of AE is connected to the capability of *S. aureus* to colonize the deeper skin layers (Niebuhr et al. 2011). Finally, *S. aureus* strains are adapted to the environment they are isolated from (Zhao et al. 2021), so this enhancement of AE growth in scratching conditions, could be due to all strains being isolated from LS skin where these strains have already adapted to an oxygen change within the skin. The likelihood of *S. aureus*

colonization within AE is higher in LS skin than NL skin (Totté et al. 2016), possibly due to AE strains flourishing in oxygen flux conditions.

4.4 *S. aureus's* Secretions Differ According to the Growth Environment

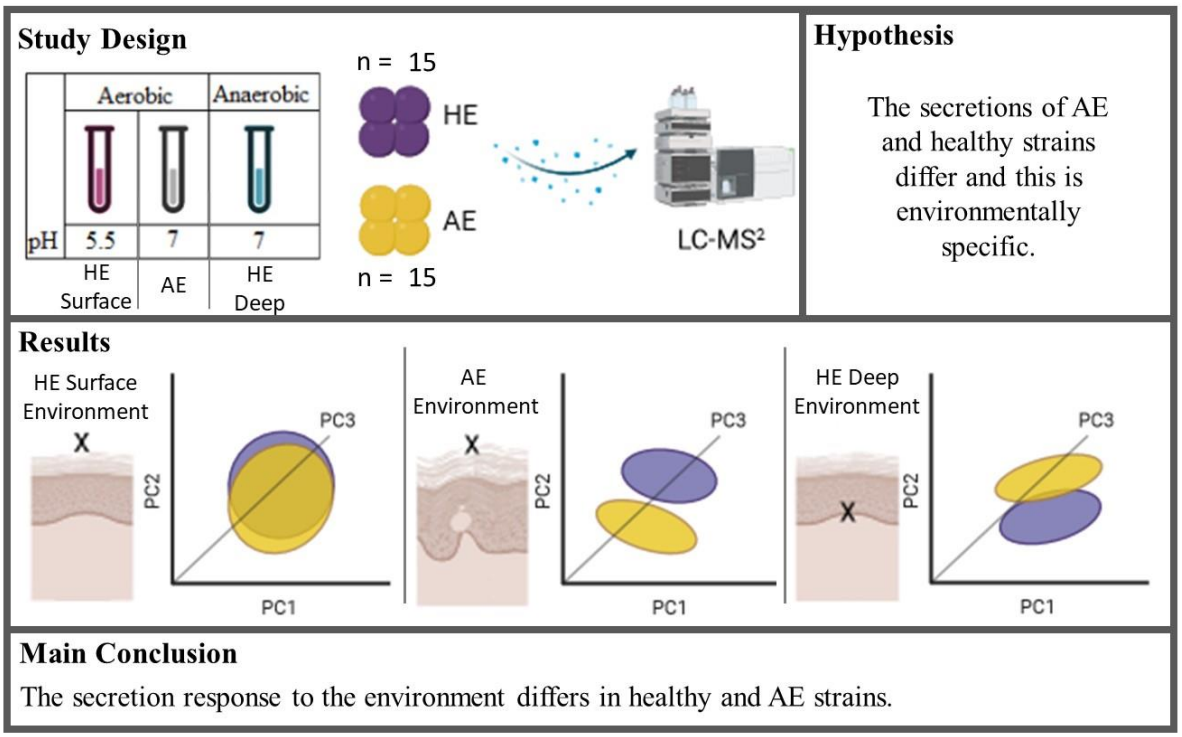


Figure 4.4 The Environment Causes Different Secretions from Healthy and AE strains

Figure created with BioRender.com. Graphical summary of the section "*S. aureus's* Secretions Differ According to the Growth Environment."

In addition to the environment influencing *S. aureus's* growth behavior, the environment can affect the metabolism of *S. aureus*. The effect of the environment on *S. aureus's* metabolism is well-known. pH 5.5, as compared to pH 7.5, can change its extracellular protein secretion profile (Weinrick et al. 2004). In response to lower pH, biofilm and virulence-related genes were lower expressed (Zhou and Fey 2020; Rode et al. 2010; Anderson et al. 2010). In addition, oxygen levels impact virulence factor regulation, such as iron (Ledala et al. 2014) and agr-based quorum sensing (Wilde et al. 2015).

Regarding secreted metabolites, i.e., the secretome, time is a major factor in the abundance of metabolites and the effect of environmental stress (Figure 3.8). Specifically, for pH-influenced changes, the stratification is best seen once *S. aureus* is within its stationary phase (Figure 3.8). With this in mind, the effects of the oxygen and pH environment on stationary phase metabolite secretions from *S. aureus* strains (n = 30) isolated from healthy and

AE individuals were measured. From this study, a greater understanding of the environmental effects on *S. aureus* in relation to AE pathogenesis can be gained.

The environment is the predominant factor differentiating the strains' secretions (Figure 3.9). When further dividing the data into each environmental group, differences between healthy and AE strains can be seen. These differences are most apparent in the pH 7.0 environment, aerobic and anaerobic (Figure 3.12). The annotated metabolites that differentiate healthy and AE strain secretions have roles relating to antimicrobials, cell adhesion, and virulence. In addition, the data was also stratified according to health status, and the environments where *S. aureus* has the lowest growth have the most similar secretion profiles according to the dendrogram (Figure 3.10). Despite this, there are unique environmental responses to the change in pH and oxygen exposure (Figure 3.11). These results show the environment's significant impact on *S. aureus* from a secretion standpoint, with many possible avenues to further explore regarding translation into clinical settings.

The environment causes substantial secretion differences in *S. aureus*. When the whole dataset is considered, the environmental differences overshadow distinctions between healthy and AE-isolated strains (Figure 3.9). ASCA analysis indicates that the metabolites contributing to the healthy versus AE secretion differences are inconsistent across all three environments due to the inability to model the interaction (Supplement Figure 7.10). This occurs because there are more subtle differences between healthy and AE strains, where only a few significant features, i.e., variations, can be detected across the two groups within each environment, and it occurs because these differences, the significant metabolites, are not consistent across all environments. This subtle change would be overshadowed in ASCA, where the whole metabolome and all conditions are considered (Camacho et al. 2023). Therefore, the data must be split into groups to decipher best healthy and AE differences.

First, the data were stratified according to the environment to view how each health status uniquely responds within the same environment. As mentioned previously in section 1.2.5, although *S. aureus* is commonly referred to as a pathogen, it can also be considered a commensal (Sakr et al. 2018), and the environment is one possible reason why healthy individuals can carry *S. aureus* and not develop AE. Within pH 7.0 environments, regardless of oxygen levels, the secretions of AE strains cluster separately from the healthy ones (Figure 3.11). At pH 5.5, such clustering is not seen, suggesting that pH may be the main switch to turn *S. aureus* from producing harmless secretions to pathogenic secretions. However, follow-up studies on translational effects regarding immune stimulation are still needed. This concept

ignores that strains could be inherently different between healthy and AE individuals from colonization and is only possible if AE strains adapt to be inhospitable in an unhealthy skin environment. Genetic and epigenetic changes can occur when a bacterium adapts to a new niche (Somerville et al. 2003), making it difficult to validate this idea if only adult strains are genetically sequenced. To validate this idea, a birth cohort closely tracing the monthly, weekly, or daily pH, and scratching, where strains are whole genome sequenced, and the pH and scratching are correlated to bacterial abundance, would need to be performed.

The metabolites with different abundances across healthy and AE strains appear to be more of an additive niche adaptation. AE strains typically have higher abundances and numbers of these metabolites that differ between the two groups (Figure 3.12). Only a portion of these metabolites can be tentatively annotated (Figure 3.11), and several of these have interesting implications for AE pathogenesis. Here we will go through those differential metabolites, organized by growth environment, and what is currently known about them. 2'-Deoxycytidine is a deoxyribonucleoside that is expressed more in healthy strains, as compared to AE, within pH 5.5 growth conditions (Figure 3.12, Supplement Table 7.2). Nucleosides can be used as antimicrobials (Serpi, Ferrari, and Pertusati 2016), and synthetic derivatives of 2'-Deoxycytidine can inhibit growth in gram-positive organisms, including methicillin-resistant *S. aureus* (MRSA) (Alexandrova et al. 2021). Among the metabolites expressed in pH 5.5, there is overlap within pH 7.0 aerobic conditions, with Acetylblasticidin S being secreted more by AE strains (Figure 3.12, Supplement Table 7.2). Acetylblasticidin S has not previously been reported to be a secretion of *S. aureus* and is produced by another gram-positive bacteria within Streptomyces (Yoshinari et al. 2013). It is an antifungal against *Aspergillus niger* (Yoshinari et al. 2013). It could be potentially used by *S. aureus* against fungal colonization, like Malassezia, a disputed skin fungal pathogen isolated from AE individuals (Faergemann 2002).

Only three health status differential metabolites secreted in anaerobic conditions could be tentatively identified (Supplement Table 7.4). Dibenzo-p-dioxin is a part of the dioxin group that can suppress the primary antibody response, and the version found in AE *S. aureus* secretions is not the strongest of the class (Figure 3.12) (Birnbaum 1994). This class has been associated with another skin disease, chloracne (Birnbaum 1994). Dibenzo-p-dioxin exposure upregulates cell mobility, secretion transport, and signal transduction genes in *Sphingomonas wittichii* (Chai et al. 2016). This could also be true for *S. aureus* but requires further study. Among gram-negative bacteria, Dibenzo-p-dioxin can be used as a carbon source (Chai et al. 2016; Hong et al. 2004), and it would be interesting to determine if genetic knockouts

preventing Dibenzo-p-dioxin production limits *S. aureus's* propagation in anaerobic conditions. 1'-Acetoxychavicol acetate is secreted by AE strains (Figure 3.12, Supplement Table 7.4) and found to be an antibacterial effective against *S. aureus* by damaging the cell membrane (Zhang et al. 2021). Treatment at non-lethal concentrations results in upregulation of the genes responsible for *S. aureus* adhesion to epithelial cells and ATP synthesis (Zhang et al. 2021). Three of the 15 AE strains did not have increased intensity for 1'-Acetoxychavicol acetate (Figure 3.12), and those three grew worse in anoxic conditions than the other AE strains (Figure 3.7). Because 1'-Acetoxychavicol acetate has been seen to upregulate ATP synthesis, it could be a potential factor for the improved growth in anaerobic conditions for AE strains. In addition, 1'-Acetoxychavicol acetate treatment can result in the clumping of *S. aureus* (Weerakkody et al. 2012). To the author's knowledge, no studies have been done to determine if this compound at sublethal doses improves biofilm formation. From the immunological side, 1'-Acetoxychavicol acetate can inhibit proinflammatory cytokine expression and attenuate the innate immune response (G. H. Ong et al. 2022). Further research should determine if this metabolite is used by *S. aureus* to evade the host's immune system. Finally, bilirubin has varying abundance between the secretions of healthy and AE strains within anaerobic conditions (Supplement Table 7.4). In patients infected with *S. aureus,* hyperbilirubinemia is common (Watanakunakorn et al. 1987), but this could be only a result of a long-term infection because, in rabbits infected with *S. aureus,* there was not a rise in serum bilirubin after 24 hrs (Quale et al. 1988). For other gram-positive bacteria, bilirubin is suggested to be an inhibitor of cellular metabolism and aerobic respiration (Nobles, Green, and Maresso 2013). As these metabolites are secreted in the anaerobic condition and more highly in AE strains (Figure 3.12), this could be a potential mechanism to explain why AE strains grow better in anaerobic conditions since this could be a self-signaling switch. This should be cross-referenced with AE nasal strains to ensure it's not a location-based effect. Also, bilirubin can be cytotoxic and disrupt the membrane of *S. aureus* (Nobles, Green, and Maresso 2013). Therefore, the proper dosage must be considered when studying it as a signaling pathway in further research. These three metabolites could explain part of the reasoning why *S. aureus,* although lower in growth in anaerobic conditions, at first glance, has differential growth between healthy and AE strains and how these strains could survive within the skin.

Finally, in the pH 7 aerobic condition, only one metabolite secreted more by healthy strains could be tentatively annotated (Figure 3.12, Supplement Table 7.3). Chlorpromazine N-oxide, unlike its parent chlorpromazine, is not a dopamine receptor blocker, but human cells

can convert it to chlorpromazine (Jaworski et al. 1993). Chlorpromazine is used to treat schizophrenia and other mental disorders (Dai et al. 2023; Thornley et al. 2003), but little is known about its use for *S. aureus*. For a few of the AE strains, there are intensities of this metabolite that are similar in level to healthy strains (Figure 3.12). Since AE is associated with mental disorders like anxiety and depression (Schonmann et al. 2020), looking at the mental states of those with these strains in a follow-up study would be interesting. Since chlorpromazine n-oxide levels are higher in these strains, it would be hypothesized that these individuals would be less likely to have a mental disorder. Several other skin resident bacteria have been associated with brain disorders like Alzheimer's, autism, and depression (Goswami et al. 2021), so the skin-brain axis is not a new concept, but novel in the sense that *S. aureus* has not yet been associated with mental disorders in AE. In a study of MRSA carriers, 33% had poor mental health (Rump et al. 2017), so chlorpromazine n-oxide is an interesting direct connection between *S. aureus* and mental health that should be explored in AE. Shikimate 3-phosphate, an intermediate within the Shikimate pathway described in *E. coli*, was secreted with higher intensity in AE strains (Figure 3.12, Supplement Table 7.3) (Herrmann and Weaver 1999; Arcuri et al. 2010). In the Shikimate pathway, shikimate is phosphorylated by shikimate kinase to produce Shikimate 3-phosphate that then later is involved with the production of chorismate, which is the precursor for aromatic amino acids (Herrmann and Weaver 1999; Arcuri et al. 2010). Consistent with our results, shikimate kinase, αroS, has been discovered within *S. aureus* (Charles 2003). The difference in Shikimate 3-phosphate intensity between healthy and AE strains (Figure 3.12) suggests a higher expression of shikimate kinase and, therefore, a subsequent buildup of Shikimate 3-phosphate. Virulence has been connected to a functioning shikimate kinase due to reduced replication (Charles 2003), and although there is no difference in growth between healthy and AE strains within the pH 7 aerobic condition (Figure 3.7, Supplement Figure 7.9), this is a new pathway for further study in AE. Chamuvaritin was also found to be secreted with higher intensities in AE strains (Figure 3.12, Supplement Table 7.3). Chamuvaritin was first isolated from *Uvaria chamae*, a plant native to Africa (Uwaifo, Okorie, and Bababunmi 1979). It was found to be mutagenic in *Salmonella typhimurium* (Uwaifo, Okorie, and Bababunmi 1979), and perhaps *S. aureus* is trying to induce self-mutagenesis for better survival in a niche they already grow well inside. Lastly, acetate was found in higher intensity among the AE strains, but a few healthy strains also expressed it in comparable levels (Figure 3.12, Supplement Table 7.3). If the secretions of *S. aureus* reflect the internal expression, then the following may be true. For *S. aureus*, acetate typically follows

increased accumulation through the exponential phase and catabolism in the post-exponential phase (Somerville et al. 2002). The secretions measured from healthy and AE strains were from cells in the post-exponential phase. Because of this, the data suggest that acetate catabolism is lower among AE strains. Other *S. aureus* strains have been reported to be catabolic mutants (Somerville et al. 2003), supporting the possibility that this could be true for AE strains. In addition, *S. aureus* without the capacity to catabolize acetate in the stationary phase has lower growth yields (Somerville et al. 2002; 2003), and for our strains, less growth is expected, but there is no difference between HE and AE strains (Supplement Figure 7.9). Therefore, another pathway in the post-exponential phase must maintain the growth efficiency without acetate catabolism, such as amino acid catabolism. Amino acids are found in higher abundance among AE skin (Ilves et al. 2021), although the abundance has not yet been correlated to *S. aureus* colonization. It may be that the AE isolates cannot catabolize acetate and take advantage of the amino acid abundance within AE skin to overgrow. Further research on correlating amino acid abundance with *S. aureus'* total abundance and confirming mutated genes related to the catabolism of acetate in AE strains is necessary to confirm this theory. Overall, the environment can influence *S. aureus'* secretions, and the metabolites highlighted above would be of interest when looking further at how *S. aureus* affects the pathogenesis of AE development.

Alongside the health status differences in secretions within an environment, it is also interesting to look across the environments within each health status. To parse out the differences across the environments in each group, the data was also stratified by health status. Like before, there is clustering among each environment and unique clustering within each environmental class (Figure 3.10). This secondary clustering was not due to nasal versus skin strain differences and must be due to extenuating factors that are yet to be determined within the strains (Figure 3.10). Based on PFGE fingerprint analysis, nasal and skin isolates are genetically very similar in the context of AE carriers (Masiuk, Wcisłek, and Jursa-Kulesza 2021). For healthy individuals, this is not fully elucidated. According to Multi-locus sequence typing (MLST), what is known is that the strains from the nose and skin are from different origins within a healthy individual (Zhao et al. 2021). Despite this, their biofilm and hemolytic activity are similar among healthy individuals, and biofilm formation was inconsistent within each MLST type (Zhao et al. 2021). This suggests that perhaps healthy nasal and skin strains are metabolically similar despite the MLST typing differences, but this can be best determined by whole genome sequencing. The secondary clustering among the secretions does not appear uniform for select strains across the three environments (Figure 3.10), which suggests unique

environmental reactions. Further study on whether these secondary clustering groups cause a different immune response within the skin would be interesting. Perhaps there are two different secretion types that strains can be further classified by, but this requires further study into how metabolites play a role in *S. aureus's* survival or adaptability.

The primary clustering is based on environment, and although the metabolites were normalized by cell number, both environments where *S. aureus* thrives the least in terms of growth capacity have the most similar secretions (Figure 3.10, Supplement Figure 7.9). When exposed to either alkalized or acidified extremes, *S. aureus* displayed a typical transcriptional response where cellular metabolism is decreased (Anderson et al. 2010), suggesting it's a survival mode response. The results in this thesis extend this thought to a survival mode for *S. aureus* secretions. At least 25% of the secretion responses to the environmental changes for healthy and AE strains are not environmentally specific (Figure 3.11). The environmental-specific secretion response (pH, O_2) is not universal across healthy and AE strains (Figure 3.11). Still, there is a 30% overlap for changing O_2 conditions and a 35% overlap in metabolites for changing pH conditions (Figure 3.11). The larger percentage of AE-specific, environmental-dependent secretions agrees with one of the hypothesized pathophysiological mechanisms for AE development, where the environment uniquely perturbates AE *S. aureus* strains, and its secretions perturb the skin and the immune system. These AE strains have a unique reaction by being more metabolically active relative to their healthy counterparts, and it would explain why some people can carry *S. aureus* but not develop AE. Virulence factors from *S. aureus* can induce AE-like inflammation in mice (Chung et al. 2022), and dysbiosis partially due to *S. aureus* drives skin inflammation (Kobayashi et al. 2015). The immune system response depends on the strain of *S. aureus*, where isolates from AE result in a more aggressive immune system response (Byrd et al. 2017). Despite the unique secretion response to different environments, these differences could be an effect of the location of isolation since the majority of the healthy strains were isolated from the nose while all of the AE strains were skin isolates. Despite this possibility, when strains were whole genome sequenced, nose and skin isolates were nearly genetically identical within an individual (personal communication on unpublished data from Dr. Reiger). In addition, although there are known environmental impacts on *S. aureus* metabolism, as described at the beginning of this section, there are limitations to using in vitro environments to test stress responses. The effect of pH stress on the metabolism appears to depend on whether the acid that reduces the medium is either strong or weak, regardless of whether the pH is equivalent (Rode et al. 2010). According to Rode et

al., at pH 4.5, the headspace of *S. aureus* can differ according to which acid is used to stress the cells (Rode et al. 2010). This shows that when analyzing metabolomics data, the in vitro environments cannot perfectly mimic the native environment because the nutritional and environmental stimuli may differ from the native environment. Furthermore, the metabolism of bacteria is different when grown on a surface or in a liquid, and since the strains normally reside on a surface, i.e. the skin, this is another limitation that must be taken into account (Dharanishanthi et al. 2021). Although there are limitations to these in vitro studies, the response to the environment appears to differ in healthy and AE strains, with exciting implications for future studies on how this translates to the immune response, virulence, and development of AE.

4.5 Translation of Metabolomics to Skin Measurements

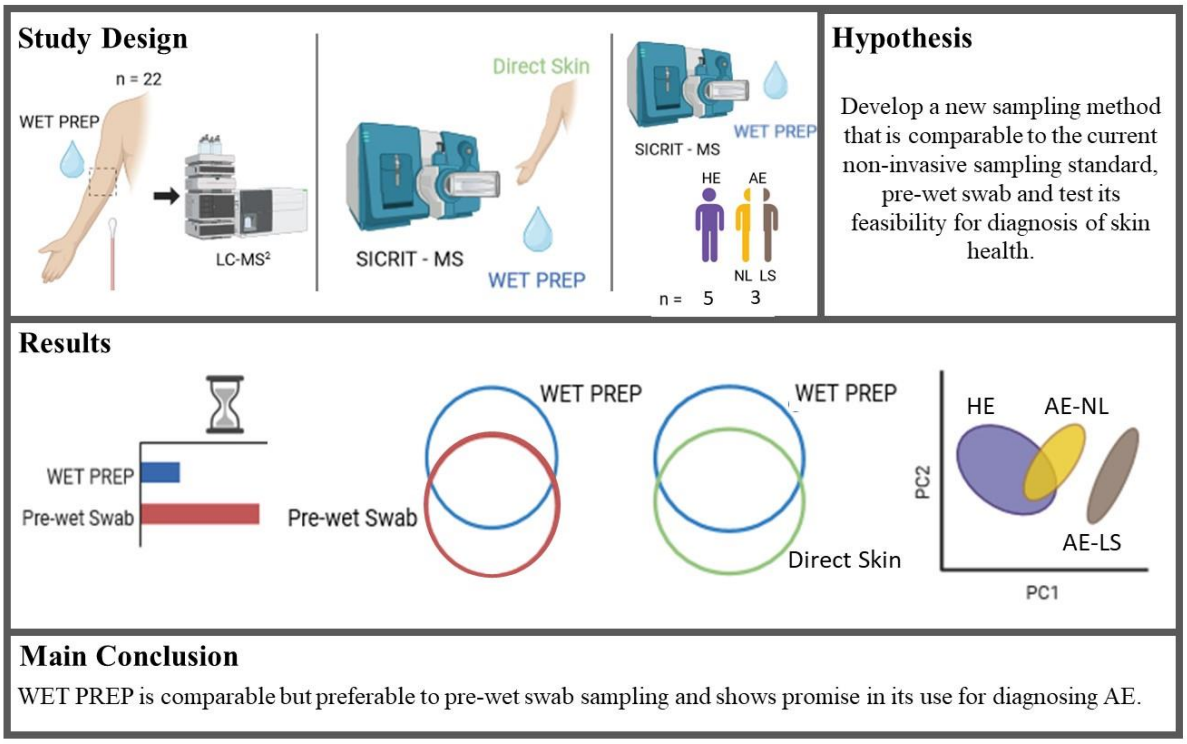


Figure 4.5 WET PREP is a Feasible Method for Skin Metabolomic Sampling

Figure created with BioRender.com. Graphical summary of the section "Translation of Metabolomics to Skin Measurements."

Alongside the observational microbiome and in vitro studies, a simpler method for clinicians and laboratory staff to capture and measure the skin metabolome was created. This is particularly important for AE because AE urgently needs objective measurement criteria (Breiteneder et al. 2020). By developing a simpler method for metabolomic sampling, metabolomics can then be applied to create a diagnostic for AE. From these pilot studies, the

developed sampling method WET PREP was found to be a feasible sampling method for skin metabolomics according to measurements from 22 individuals. This is due to its good coverage and capture of the skin metabolome (Figure 3.14). The results from this study also brought attention to the fact that the sampling method can impact skin metabolome measurements, which wasn't known nor tested previously (Figure 3.14, Table 3.1). In addition, WET PREP successfully captured not only stable metabolites but also VOCs with high overlap in coverage as compared to direct skin measurements (Figure 3.17). The VOCs, by WET PREP, can also, based on preliminary tests, distinguish AE samples from healthy (Figure 3.18). From these studies, it appears that WET PREP could help with the advancement of creating skin metabolome diagnostics, pending confirmation with a larger cohort that contains AE individuals.

As covered previously in section 1.3.1, there are various methods for capturing the skin metabolome. The non-invasive standard, pre-wet swab, requires too much time pre- and post-processing to be a clinically feasible method. Because of this, WET PREP was created with results published in (Afghani et al. 2021). Briefly, this study found that except for lipids, the metabolite detection in WET PREP does compare to the universal standard (Figure 3.14). It was also determined that the method for collecting skin metabolome highly influences the metabolome collected, and depending on the metabolites of interest, different methods should be used (Table 3.1). WET PREP is reliable for comparing bilateral replicates (Figure 3.15), often done in skin studies, and can collect an extensive range of metabolites (Figure 3.14, Table 3.1). This is beneficial because WET PREP is non-invasive, requires no pre-preparation, and is comparable, except lipid detection, to the conventional non-invasive sampling method, pre-wet swabs.

Following this study, a pilot study was performed to see if WET PREP could detect VOCs (Figure 3.17) where five healthy and three AE individuals were sampled. VOCs are only one part of the small molecules within the skin, with the metabolome representing the lipids, amino acids, VOCs, and other small molecules. VOCs can be smelt, and this odor can be used to train dogs to successfully and specifically detect cancer (Yoel et al. 2015). There is a scent of AE, and MRSA has been suggested as the cause for the smell of AE (Ellis et al. 2008). Although MRSA could be the leading cause of the scent, the microbiome is highly correlated with the VOCs of the skin (Dormont, Bessière, and Cohuet 2013). VOCs have been shown to affect the risk of AE development (Kwon et al. 2015), but no studies have tested using VOCs secreted from AE skin as a diagnostic tool. Therefore, the feasibility of WET PREP in detecting

the VOCs of the skin was tested, and the compounds detected had a high overlap with those detected directly from the skin (Figure 3.17). In addition, VOCs measured from the skin of AE individuals by WET PREP do separate according to PCA (Figure 3.18), leading to the concept that VOCs could be used to develop a diagnosis for AE. This study is limited, though, to the low participant numbers. A study with a larger n would need to be performed to validate these results. Afterward, the metabolites detected should be cross-referenced to online databases for tentative annotation following confirmation with standards, especially standards of known skin VOCs. Nonetheless, these results provide a positive outlook for using WET PREP in the context of measuring skin metabolome and the diagnosis of AE, and larger follow-up studies should be performed.

Chapter 5 - Conclusion

5.1 Summary

The thesis aimed to provide new information regarding factors involved with skin homeostasis and, in turn, its impact on AE, along with developing new methods to measure skin health, i.e., diagnose skin disease. From this investigation, the following points were discovered. Bacteria can be detected throughout the layers of the skin and a higher bacterial load is present in AE. In addition, although Staphylococci species are diverse in pathogenicity, Staphylococci load does correlate to local EASI. In addition, *S. aureus* was only found present in AE, with its DNA found across the layers of AE LS skin. In addition, to researching the presence of bacteria across the layers of the skin, selected biomes were also explored. According to next-generation sequencing, the anaerobic microbiome is present in the skin and has decreased relative abundance in AE. More specifically, the relative abundance of the anaerobic microbiome decreases with increasing AE severity. These studies showed how the quantities of the microbiome change within AE.

Alongside exploring the microbiome, *S. aureus*, which is overgrown in AE, was of particular focus. Both oxygen and pH were found to be influential to *S. aureus*. Scratching conditions, i.e., oxygen flux, can cause *S. aureus* to “bloom” in growth. The environmental impacts are also not limited to growth changes. The environment impacts the secretions of *S. aureus*, and both the response to the environment and secretions within the environment are different between healthy and AE strains. This work highlights the importance of the environment in the pathogenesis of AE, specifically for those colonized with *S. aureus*.

Along with bacterial and environmental influences, developing a better sampling method for future AE diagnostic creation was important. The comparison of WET PREP to pre-wet swab sampling showed that the sampling method influences the detection of metabolites on the skin. Besides lipid detection, WET PREP is comparable to pre-wet swab sampling but preferable because of its simpler processing steps. In addition, through its application of capturing VOCs, WET PREP shows promise in its use for skin metabolomics. Overall, the studies within this work highlight how the environment is an important factor in AE pathogenesis and provide an outlook for future metabolome sampling.

5.2 Implications and Future Avenues for Investigation or Perspectives

This work explored the impacts of the environment (microbial and chemical) on skin homeostasis and its relation to AE. Throughout Chapter 4, many potential avenues for future research have been suggested. Broadly, the impact of this work highlights the importance of considering the skin as a whole and not only on the surface. This study is the first, where several different depths are sampled across three known skin diseases. The depth bacteria can reach causes different immunological responses (Ogonowska et al. 2021), and with regards to future studies on the immunological impact of bacteria in skin disease, epi-cutaneous versus intradermal challenges must accurately reflect the actual location of the bacteria within the skin. The research done here begins to answer this, but further studies on larger cohorts and targeting more bacteria of interest in the skin would be needed. In addition, this work shows that when the skin has known barrier impairments, it is overburdened in terms of bacterial load. This shows a commonality between AE and psoriasis, despite the typically higher AMP response among psoriasis. Restoring the barrier is a good first step for these patients before pro- or pre-biotic therapy. Otherwise, the treatment encouraging microbial growth could further overburden the skin. Studies on the upregulation of immune factors like AMP or a specific cytokine signature in correlation to bacterial abundance should be performed to confirm this overburden within the skin. The importance of the anaerobic microbiome in AE development has long been considered, and this work is the first to demonstrate that the anaerobic microbiome is decreased in AE in both lesional and non-lesional skin. Combined with the knowledge that *S. aureus* can take advantage of the changing oxygen niche; this signals even more that restoring the skin barrier is a critical point for therapy. Restoring the oxygen differential could follow a similar procedure to hyperbaric pressure therapy, but care must also be taken to remove *S. aureus* from the skin prior. After the removal of *S. aureus*, the affected

area could be placed under an anaerobic jar, much like the portable ones used for culturing anaerobes. This work also confirms and elaborates on how the environment can play a role in the pathogenesis of the *S. aureus* endotype of AE. It brings several metabolites into the limelight for further targeted studies on their roles and potential as therapeutics. Finally, this work has generated a new method for sampling in skin metabolomics and shown its potential for application for diagnosis of AE with the application of this method in the ProRaD cohort underway.

Chapter 6 - Bibliography

A Page from the Ebers Papyrus, Written circa 1500 B.C. n.d. https://wellcomeimages.org/indexplus/obf_images/ab/f9/14b2986971407c3d997ee0c643c0.jpg Gallery: https://wellcomeimages.org/indexplus/image/M0008455.html Wellcome Collection gallery (2018-03-22): https://wellcomecollection.org/works/yzg97evz CC-BY-4.0. Accessed September 5, 2023. https://commons.wikimedia.org/wiki/File:A_page_from_the_Ebers_Papyrus,_written_circa_1500_B.C._Wellcome_M0008455.jpg?uselang=pt-br.

Acker, Karen P., Tania Wong Fok Lung, Emily West, Joshua Craft, Apurva Narechania, Hannah Smith, Kelsey O'Brien, et al. 2019. "Strains of Staphylococcus Aureus That Colonize and Infect Skin Harbor Mutations in Metabolic Genes." *iScience* 19 (September): 281–90. https://doi.org/10.1016/j.isci.2019.07.037.

Afghani, Jamie, Claudia Huelpuesch, Matthias Reiger, Philippe Schmitt-Kopplin, Claudia Traidl-Hoffmann, and Constanze Mueller. 2021. "Enhanced Access to the Health-Related Skin Metabolome by Fast, Reproducible and Non-Invasive Wet-Prep Sampling."

Afghani, Jamie, Claudia Traidl-Hoffmann, Philippe Schmitt-Kopplin, Matthias Reiger, and Constanze Mueller. 2022. "An Overview of the Latest Metabolomics Studies on Atopic Eczema with New Directions for Study." *International Journal of Molecular Sciences* 23 (15): 8791. https://doi.org/10.3390/ijms23158791.

Aggarwal, Nikhil, Shohei Kitano, Ginette Ru Ying Puah, Sandra Kittelmann, In Young Hwang, and Matthew Wook Chang. 2022. "Microbiome and Human Health: Current Understanding, Engineering, and Enabling Technologies." *Chemical Reviews* 123 (1): 31–72. https://doi.org/10.1021/acs.chemrev.2c00431.

Agrawal, Rachana, and Judith A. Woodfolk. 2014. "Skin Barrier Defects in Atopic Dermatitis." *Current Allergy and Asthma Reports* 14 (5): 433. https://doi.org/10.1007/s11882-014-0433-9.

Ahn, Kangmo. 2014. "The Role of Air Pollutants in Atopic Dermatitis." *The Journal of Allergy and Clinical Immunology* 134 (5): 993–99; discussion 1000. https://doi.org/10.1016/j.jaci.2014.09.023.

Al Kindi, Arwa, Abdullah M. Alkahtani, Mayimuna Nalubega, Cecile El-Chami, Catherine O'Neill, Peter D. Arkwright, and Joanne L. Pennock. 2019. "Staphylococcus Aureus Internalized by Skin Keratinocytes Evade Antibiotic Killing." *Frontiers in Microbiology* 10 (September). https://doi.org/10.3389/fmicb.2019.02242.

Chapter 6 -Bibliography

Alexandrova, Liudmila A., Maxim V. Jasko, Sergey D. Negrya, Pavel N. Solyev, Oleg V. Shevchenko, Andrei P. Solodinin, Daria P. Kolonitskaya, et al. 2021. "Discovery of Novel N4-Alkylcytidines as Promising Antimicrobial Agents." *European Journal of Medicinal Chemistry* 215 (April): 113212. https://doi.org/10.1016/j.ejmech.2021.113212.

Ali, Saba M., and Gil Yosipovitch. 2013. "Skin pH: From Basic Science to Basic Skin Care." *Acta Dermato-Venereologica* 93 (3): 261–67. https://doi.org/10.2340/00015555-1531.

Altunbulakli, Can, Matthias Reiger, Avidan U. Neumann, Natalie Garzorz-Stark, Megan Fleming, Claudia Huelpuesch, Francesc Castro-Giner, Kilian Eyerich, Cezmi A. Akdis, and Claudia Traidl-Hoffmann. 2018. "Relations between Epidermal Barrier Dysregulation and Staphylococcus Species-Dominated Microbiome Dysbiosis in Patients with Atopic Dermatitis." *The Journal of Allergy and Clinical Immunology* 142 (5): 1643-1647.e12. https://doi.org/10.1016/j.jaci.2018.07.005.

Anderson, Kelsi L., Christelle M. Roux, Matthew W. Olson, Thanh T. Luong, Chia Y. Lee, Robert Olson, and Paul M. Dunman. 2010. "Characterizing the Effects of Inorganic Acid and Alkaline Shock on the Staphylococcus Aureus Transcriptome and Messenger RNA Turnover." *FEMS Immunology and Medical Microbiology* 60 (3): 208–50. https://doi.org/10.1111/j.1574-695X.2010.00736.x.

Ansel, John C., Cheryl A. Armstrong, InSung Song, Kimberly L. Quinlan, John E. Olerud, S. Wright Caughman, and Nigel W. Bunnett. 1997. "Interactions of the Skin and Nervous System." *Journal of Investigative Dermatology Symposium Proceedings* 2 (1): 23–26. https://doi.org/10.1038/jidsymp.1997.6.

Arcuri, Helen, Geraldo Zafalon, Evandro Marucci, Carlos Bonalumi, Nelson Silveira, José Machado, Walter De Azevedo Jr, and Mário Palma. 2010. "SKPDB: A Structural Database of Shikimate Pathway Enzymes." *BMC Bioinformatics* 11 (January): 12. https://doi.org/10.1186/1471-2105-11-12.

Arikawa, Junko, Mutsumi Ishibashi, Makoto Kawashima, Yutaka Takagi, Yoshiaki Ichikawa, and Genji Imokawa. 2002. "Decreased Levels of Sphingosine, a Natural Antimicrobial Agent, May Be Associated with Vulnerability of the Stratum Corneum from Patients with Atopic Dermatitis to Colonization by Staphylococcus Aureus." *The Journal of Investigative Dermatology* 119 (2): 433–39. https://doi.org/10.1046/j.1523-1747.2002.01846.x.

Armstrong-Esther, C. A. 1976. "Carriage Patterns of Staphylococcus Aureus in a Healthy Non-Hospital Population of Adults and Children." *Annals of Human Biology* 3 (3): 221–27. https://doi.org/10.1080/03014467600001381.

Arndt, Jenna, Nananamibia Smith, and Francisco Tausk. 2008. "Stress and Atopic Dermatitis." *Current Allergy and Asthma Reports* 8 (4): 312–17. https://doi.org/10.1007/s11882-008-0050-6.

Auerswald, Steffen, Stephan Schreml, Robert Meier, Alexandra Blancke Soares, Maximilian Niyazi, Sebastian Marschner, Claus Belka, Martin Canis, and Frank Haubner. 2019. "Wound Monitoring of pH and Oxygen in Patients after Radiation Therapy." *Radiation Oncology* 14 (1): 199. https://doi.org/10.1186/s13014-019-1413-y.

Azulay, Rubem David. 2003. "Nascimento da Dermatologia." *Anais Brasileiros de Dermatologia* 78 (October): 615–17. https://doi.org/10.1590/S0365-05962003000500012.

Bansal, Ciara Jade, and Amolak Singh Bansal. 2019. "Stress, Pseudoallergens, Autoimmunity, Infection and Inflammation in Chronic Spontaneous Urticaria." *Allergy, Asthma & Clinical Immunology* 15 (1): 56. https://doi.org/10.1186/s13223-019-0372-z.

Chapter 6 -Bibliography

Bantz, Selene K., Zhou Zhu, and Tao Zheng. 2014. "The Atopic March: Progression from Atopic Dermatitis to Allergic Rhinitis and Asthma." *Journal of Clinical & Cellular Immunology* 5 (2): 202. https://doi.org/10.4172/2155-9899.1000202.

Barnes, Christopher J., Maja-Lisa Clausen, Maria Asplund, Linett Rasmussen, Caroline Meyer Olesen, Yasemin Topal Yüsel, Paal Skytt Andersen, Thomas Litman, Anders Johannes Hansen, and Tove Agner. 2022. "Temporal and Spatial Variation of the Skin-Associated Bacteria from Healthy Participants and Atopic Dermatitis Patients." *mSphere* 7 (1): e00917-21. https://doi.org/10.1128/msphere.00917-21.

Barnes, Kathleen C. 2010. "An Update on the Genetics of Atopic Dermatitis: Scratching the Surface in 2009." *The Journal of Allergy and Clinical Immunology* 125 (1): 16-29.e1-11; quiz 30–31. https://doi.org/10.1016/j.jaci.2009.11.008.

Bay, Lene, Christopher James Barnes, Blaine Gabriel Fritz, Jonathan Thorsen, Marlene Elise Møller Restrup, Linett Rasmussen, Johan Kløvgaard Sørensen, et al. 2020. "Universal Dermal Microbiome in Human Skin." *mBio* 11 (1): e02945-19. https://doi.org/10.1128/mBio.02945-19.

Benediktsdóttir, Eva, and Anna Hambraeus. 1982. "Dispersal of Non-Sporeforming Anaerobic Bacteria from the Skin." *Epidemiology & Infection* 88 (3): 487–500. https://doi.org/10.1017/S0022172400070340.

Berg, Gabriele, Daria Rybakova, Doreen Fischer, Tomislav Cernava, Marie-Christine Champomier Vergès, Trevor Charles, Xiaoyulong Chen, et al. 2020. "Microbiome Definition Re-Visited: Old Concepts and New Challenges." *Microbiome* 8 (1): 103. https://doi.org/10.1186/s40168-020-00875-0.

Besten, Gijs den, Karen van Eunen, Albert K. Groen, Koen Venema, Dirk-Jan Reijngoud, and Barbara M. Bakker. 2013. "The Role of Short-Chain Fatty Acids in the Interplay between Diet, Gut Microbiota, and Host Energy Metabolism." *Journal of Lipid Research* 54 (9): 2325–40. https://doi.org/10.1194/jlr.R036012.

Bhattacharya, Tanya, Mark A. Strom, and Peter A. Lio. 2016. "Historical Perspectives on Atopic Dermatitis: Eczema Through the Ages." *Pediatric Dermatology* 33 (4): 375–79. https://doi.org/10.1111/pde.12853.

Bhattacharyya, M., M. Reiger, L. Rauer, C. Huelpuesch, C. Traidl-Hoffmann, and Avidan Neumann. 2019. "AnnotIEM: A Novel Algorithm for Species Level Annotation of 16S Gene Based Microbial OTUs." 8 (1660). https://doi.org/10.7490/f1000research.1117510.1.

Bieber, T., C. Akdis, R. Lauener, C. Traidl-Hoffmann, P. Schmid-Grendelmeier, G. Schäppi, J.-P. Allam, et al. 2016. "Global Allergy Forum and 3rd Davos Declaration 2015: Atopic Dermatitis/Eczema: Challenges and Opportunities toward Precision Medicine." *Allergy* 71 (5): 588–92. https://doi.org/10.1111/all.12857.

Bieber, Thomas, Claudia Traidl-Hoffmann, Georg Schäppi, Roger Lauener, Cezmi Akdis, and Peter Schmid-Grendlmeier. 2020. "Unraveling the Complexity of Atopic Dermatitis: The CK-CARE Approach toward Precision Medicine." *Allergy*, January. https://doi.org/10.1111/all.14194.

Birnbaum, L S. 1994. "The Mechanism of Dioxin Toxicity: Relationship to Risk Assessment." *Environmental Health Perspectives* 102 (Suppl 9): 157–67.

Bjerre, R. D., J. Bandier, L. Skov, L. Engstrand, and J. D. Johansen. 2017. "The Role of the Skin Microbiome in Atopic Dermatitis: A Systematic Review." *The British Journal of Dermatology* 177 (5): 1272–78. https://doi.org/10.1111/bjd.15390.

Bjerre, Rie Dybboe, Jacob Bak Holm, Albert Palleja, Julie Sølberg, Lone Skov, and Jeanne Duus Johansen. 2021. "Skin Dysbiosis in the Microbiome in Atopic Dermatitis Is Site-Specific and Involves Bacteria, Fungus and Virus." *BMC Microbiology* 21 (1): 256. https://doi.org/10.1186/s12866-021-02302-2.

Chapter 6 -Bibliography

Black, Antony P. B., Michael R. Ardern-Jones, Victoria Kasprowicz, Paul Bowness, Louise Jones, Abigail S. Bailey, and Graham S. Ogg. 2007. "Human Keratinocyte Induction of Rapid Effector Function in Antigen-Specific Memory CD4+ and CD8+ T Cells." *European Journal of Immunology* 37 (6): 1485–93. https://doi.org/10.1002/eji.200636915.

Boehncke, Wolf-Henning, and Michael P. Schön. 2015. "Psoriasis." *Lancet (London, England)* 386 (9997): 983–94. https://doi.org/10.1016/S0140-6736(14)61909-7.

Bouslimani, Amina, Carla Porto, Christopher M. Rath, Mingxun Wang, Yurong Guo, Antonio Gonzalez, Donna Berg-Lyon, et al. 2015. "Molecular Cartography of the Human Skin Surface in 3D." *Proceedings of the National Academy of Sciences* 112 (17): E2120–29. https://doi.org/10.1073/pnas.1424409112.

Bouslimani, Amina, Ricardo da Silva, Tomasz Kosciolek, Stefan Janssen, Chris Callewaert, Amnon Amir, Kathleen Dorrestein, et al. 2019. "The Impact of Skin Care Products on Skin Chemistry and Microbiome Dynamics." *BMC Biology* 17 (June). https://doi.org/10.1186/s12915-019-0660-6.

Breiteneder, Heimo, Ya-Qi Peng, Ioana Agache, Zuzana Diamant, Thomas Eiwegger, Wytske J. Fokkens, Claudia Traidl-Hoffmann, et al. 2020. "Biomarkers for Diagnosis and Prediction of Therapy Responses in Allergic Diseases and Asthma." *Allergy* 75 (12): 3039–68. https://doi.org/10.1111/all.14582.

Breuer, K., S. HAussler, A. Kapp, and T. Werfel. 2002. "Staphylococcus Aureus: Colonizing Features and Influence of an Antibacterial Treatment in Adults with Atopic Dermatitis." *The British Journal of Dermatology* 147 (1): 55–61. https://doi.org/10.1046/j.1365-2133.2002.04872.x.

Brinke, Anke Schulte to, Antje Mehlich, Claudia Doberenz, and Ciska Janssens-Böcker. 2021. "Acidification of the Skin and Maintenance of the Physiological Skin pH Value by Buffered Skin Care Products Formulated around pH 4." *Journal of Cosmetics, Dermatological Sciences and Applications* 11 (1): 44–57. https://doi.org/10.4236/jcdsa.2021.111005.

Brown, Morgan M., and Alexander R. Horswill. 2020. "Staphylococcus Epidermidis—Skin Friend or Foe?" *PLoS Pathogens* 16 (11): e1009026. https://doi.org/10.1371/journal.ppat.1009026.

Brown, Sara J., and W. H. Irwin McLean. 2012. "One Remarkable Molecule: Filaggrin." *The Journal of Investigative Dermatology* 132 (3 Pt 2): 751–62. https://doi.org/10.1038/jid.2011.393.

Bucks, Daniel A. W. 1984. "Skin Structure and Metabolism: Relevance to the Design of Cutaneous Therapeutics." *Pharmaceutical Research* 1 (4): 148–53. https://doi.org/10.1023/A:1016340423079.

Byers, Robert A., Raman Maiti, Simon G. Danby, Elaine J. Pang, Bethany Mitchell, Matt J. Carré, Roger Lewis, Michael J. Cork, and Stephen J. Matcher. 2018. "Sub-Clinical Assessment of Atopic Dermatitis Severity Using Angiographic Optical Coherence Tomography." *Biomedical Optics Express* 9 (4): 2001–17. https://doi.org/10.1364/BOE.9.002001.

Byrd, Allyson L., Clay Deming, Sara K.B. Cassidy, Oliver J. Harrison, Weng-Ian Ng, Sean Conlan, Yasmine Belkaid, Julia A. Segre, and Heidi H. Kong. 2017. "Staphylococcus Aureus and S. Epidermidis Strain Diversity Underlying Human Atopic Dermatitis." *Science Translational Medicine* 9 (397). https://doi.org/10.1126/scitranslmed.aal4651.

Calderón-Santiago, Mónica, Feliciano Priego-Capote, Natacha Turck, Xavier Robin, Bernabé Jurado-Gámez, Jean C. Sanchez, and María D. Luque de Castro. 2015. "Human Sweat Metabolomics for Lung Cancer Screening." *Analytical and Bioanalytical Chemistry* 407 (18): 5381–92. https://doi.org/10.1007/s00216-015-8700-8.

Chapter 6 -Bibliography

Camacho, José, Raffaele Vitale, David Morales-Jiménez, and Carolina Gómez-Llorente. 2023. "Variable-Selection ANOVA Simultaneous Component Analysis (VASCA)." *Bioinformatics* 39 (1): btac795. https://doi.org/10.1093/bioinformatics/btac795.

Cardona, Ivan D., Sang Hyun Cho, and Donald Y. M. Leung. 2006. "Role of Bacterial Superantigens in Atopic Dermatitis : Implications for Future Therapeutic Strategies." *American Journal of Clinical Dermatology* 7 (5): 273–79. https://doi.org/10.2165/00128071-200607050-00001.

Chai, Benli, Tamara V. Tsoi, Shoko Iwai, Cun Liu, Jordan A. Fish, Cheng Gu, Timothy A. Johnson, et al. 2016. "Sphingomonas Wittichii Strain RW1 Genome-Wide Gene Expression Shifts in Response to Dioxins and Clay." *PLoS ONE* 11 (6): e0157008. https://doi.org/10.1371/journal.pone.0157008.

Charles, Ian George. 2003. Shikimate kinase from s. aureus for drug discovery. World Intellectual Property Organization WO2003012129A2, filed July 25, 2002, and issued February 13, 2003. https://patents.google.com/patent/WO2003012129A2/en.

Chen, Y. Erin, Michael A. Fischbach, and Yasmine Belkaid. 2018. "Skin Microbiota–Host Interactions." *Nature* 553 (7689): 427–36. https://doi.org/10.1038/nature25177.

Chiu, Isaac M., Balthasar A. Heesters, Nader Ghasemlou, Christian A. Von Hehn, Fan Zhao, Johnathan Tran, Brian Wainger, et al. 2013. "Bacteria Activate Sensory Neurons That Modulate Pain and Inflammation." *Nature* 501 (7465): 52–57. https://doi.org/10.1038/nature12479.

Choi, Eung-Ho, Mao-Qiang Man, Pu Xu, Shujun Xin, Zhili Liu, Debra A. Crumrine, Yan J. Jiang, et al. 2007. "Stratum Corneum Acidification Is Impaired in Moderately Aged Human and Murine Skin." *The Journal of Investigative Dermatology* 127 (12): 2847–56. https://doi.org/10.1038/sj.jid.5700913.

Chong, Jasmine, David S. Wishart, and Jianguo Xia. 2019. "Using MetaboAnalyst 4.0 for Comprehensive and Integrative Metabolomics Data Analysis." *Current Protocols in Bioinformatics* 68 (1): e86. https://doi.org/10.1002/cpbi.86.

Chovatiya, R., and J.I. Silverberg. 2022. "The Heterogeneity of Atopic Dermatitis." *Journal of Drugs in Dermatology : JDD* 21 (2): 172–76. https://doi.org/10.36849/JDD.6408.

Chung, Ethan Jachen, Chia-Hui Luo, Christina Li-Ping Thio, and Ya-Jen Chang. 2022. "Immunomodulatory Role of Staphylococcus Aureus in Atopic Dermatitis." *Pathogens* 11 (4): 422. https://doi.org/10.3390/pathogens11040422.

Clausen, M.-L., S. M. Edslev, P. S. Andersen, K. Clemmensen, K. A. Krogfelt, and T. Agner. 2017. "Staphylococcus Aureus Colonization in Atopic Eczema and Its Association with Filaggrin Gene Mutations." *The British Journal of Dermatology* 177 (5): 1394–1400. https://doi.org/10.1111/bjd.15470.

Cookson, William O. C. M., and Miriam F. Moffatt. 2002. "The Genetics of Atopic Dermatitis." *Current Opinion in Allergy and Clinical Immunology* 2 (5): 383–87. https://doi.org/10.1097/00130832-200210000-00003.

Cornbleet, T., and N. R. Joseph. 1954. "Recovery of Normal and Eczematous Skin from Alkali; Control of Surface pH by Exchange Resins." *The Journal of Investigative Dermatology* 23 (6): 455–61. https://doi.org/10.1038/jid.1954.127.

Costello, Elizabeth K., Christian L. Lauber, Micah Hamady, Noah Fierer, Jeffrey I. Gordon, and Rob Knight. 2009. "Bacterial Community Variation in Human Body Habitats Across Space and Time." *Science (New York, N.Y.)* 326 (5960): 1694–97. https://doi.org/10.1126/science.1177486.

Czarnowicki, Tali, Helen He, James G. Krueger, and Emma Guttman-Yassky. 2019. "Atopic Dermatitis Endotypes and Implications for Targeted Therapeutics." *Journal of Allergy and Clinical Immunology* 143 (1): 1–11. https://doi.org/10.1016/j.jaci.2018.10.032.

Daëron, Marc. 2022. "The Immune System as a System of Relations." *Frontiers in Immunology* 13. https://www.frontiersin.org/articles/10.3389/fimmu.2022.984678.

Dai, Jinxia, Hui Lin, Yuning Pan, Yanmin Sun, Ye Wang, Jun-Qin Qiao, Hong-Zhen Lian, and Chun-Xiang Xu. 2023. "Determination of Chlorpromazine and Its Metabolites in Animal-Derived Foods Using QuEChERS-Based Extraction, EMR-Lipid Cleanup, and UHPLC-Q-Orbitrap MS Analysis." *Food Chemistry* 403 (March): 134298. https://doi.org/10.1016/j.foodchem.2022.134298.

Dand, Nick, Satveer K. Mahil, Francesca Capon, Catherine H. Smith, Michael A. Simpson, and Jonathan N. Barker. 2020. "Psoriasis and Genetics." *Acta Dermato-Venereologica* 100 (3): 5647. https://doi.org/10.2340/00015555-3384.

De Tomassi, Amedeo, Anna Reiter, Matthias Reiger, Luise Rauer, Robin Rohayem, CK-CARE Study Group, Claudia Traidl-Hoffmann, Avidan U. Neumann, and Claudia Hülpüsch. 2023. "Combining 16S Sequencing and qPCR Quantification Reveals Staphylococcus Aureus Driven Bacterial Overgrowth in the Skin of Severe Atopic Dermatitis Patients." *Biomolecules* 13 (7): 1030. https://doi.org/10.3390/biom13071030.

Demessant-Flavigny, Anne-Laure, Sophie Connétable, Delphine Kerob, Magali Moreau, Luc Aguilar, and Andreas Wollenberg. 2023. "Skin Microbiome Dysbiosis and the Role of Staphylococcus Aureus in Atopic Dermatitis in Adults and Children: A Narrative Review." *Journal of the European Academy of Dermatology and Venereology: JEADV* 37 Suppl 5 (June): 3–17. https://doi.org/10.1111/jdv.19125.

Dharanishanthi, Veeramuthu, Amit Orgad, Neta Rotem, Efrat Hagai, Jeny Kerstnus-Banchik, Julius Ben-Ari, Tim Harig, Srinivasa Rao Ravella, Stefan Schulz, and Yael Helman. 2021. "Bacterial-Induced pH Shifts Link Individual Cell Physiology to Macroscale Collective Behavior." *Proceedings of the National Academy of Sciences of the United States of America* 118 (14): e2014346118. https://doi.org/10.1073/pnas.2014346118.

Dormont, Laurent, Jean-Marie Bessière, and Anna Cohuet. 2013. "Human Skin Volatiles: A Review." *Journal of Chemical Ecology* 39 (5): 569–78. https://doi.org/10.1007/s10886-013-0286-z.

Dubin, Celina, Ester Del Duca, and Emma Guttman-Yassky. 2021. "The IL-4, IL-13 and IL-31 Pathways in Atopic Dermatitis." *Expert Review of Clinical Immunology* 17 (8): 835–52. https://doi.org/10.1080/1744666X.2021.1940962.

Dwyer, Laura R., and Tiffany C. Scharschmidt. 2022. "Early Life Host-Microbe Interactions in Skin." *Cell Host & Microbe* 30 (5): 684–95. https://doi.org/10.1016/j.chom.2022.02.016.

Elias, Peter M. 1996. "Stratum Corneum Architecture, Metabolic Activity and Interactivity with Subjacent Cell Layers." *Experimental Dermatology* 5 (4): 191–201. https://doi.org/10.1111/j.1600-0625.1996.tb00117.x.

Ellis, Kam-Lun, Alexander K. C. Leung, Albert Y. F. Kong, Ting-fan Leung, and Margaret Ip. 2008. "Atopic Dermatitis Complicated by Methicillin-Resistant Staphylococcus Aureus Infection." *Journal of the National Medical Association* 100 (7): 797–801. https://doi.org/10.1016/S0027-9684(15)31373-0.

Elpa, Decibel P., Hsien-Yi Chiu, Shu-Pao Wu, and Pawel L. Urban. 2021. "Skin Metabolomics." *Trends in Endocrinology & Metabolism* 32 (2): 66–75. https://doi.org/10.1016/j.tem.2020.11.009.

Emerson, Joanne B., Rachel I. Adams, Clarisse M. Betancourt Román, Brandon Brooks, David A. Coil, Katherine Dahlhausen, Holly H. Ganz, et al. 2017. "Schrödinger's Microbes: Tools for Distinguishing the Living from the Dead in Microbial Ecosystems." *Microbiome* 5 (1): 86. https://doi.org/10.1186/s40168-017-0285-3.

Evans, Sydney M., Amy E. Schrlau, Ara A. Chalian, Paul Zhang, and Cameron J. Koch. 2006. "Oxygen Levels in Normal and Previously Irradiated Human Skin as Assessed by EF5

Binding." *Journal of Investigative Dermatology* 126 (12): 2596–2606. https://doi.org/10.1038/sj.jid.5700451.

Everett, M. A. 1979. "Anne-Charles Lorry. The First French Dermatologist." *International Journal of Dermatology* 18 (9): 762–64. https://doi.org/10.1111/j.1365-4362.1979.tb05018.x.

Eyerich, Stefanie, Kilian Eyerich, Claudia Traidl-Hoffmann, and Tilo Biedermann. 2018. "Cutaneous Barriers and Skin Immunity: Differentiating A Connected Network." *Trends in Immunology* 39 (4): 315–27. https://doi.org/10.1016/j.it.2018.02.004.

Faergemann, Jan. 2002. "Atopic Dermatitis and Fungi." *Clinical Microbiology Reviews* 15 (4): 545–63. https://doi.org/10.1128/CMR.15.4.545-563.2002.

Feingold, Kenneth R., and Peter M. Elias. 2014. "Role of Lipids in the Formation and Maintenance of the Cutaneous Permeability Barrier." *Biochimica Et Biophysica Acta* 1841 (3): 280–94. https://doi.org/10.1016/j.bbalip.2013.11.007.

Ferreira, Iago Gonçalves, Magda Blessmann Weber, and Renan Rangel Bonamigo. 2021. "History of Dermatology: The Study of Skin Diseases over the Centuries." *Anais Brasileiros de Dermatologia* 96 (3): 332–45. https://doi.org/10.1016/j.abd.2020.09.006.

Feuillie, Cécile, Pauline Vitry, Maeve A. McAleer, Sanja Kezic, Alan D. Irvine, Joan A. Geoghegan, and Yves F. Dufrêne. 2018. "Adhesion of Staphylococcus Aureus to Corneocytes from Atopic Dermatitis Patients Is Controlled by Natural Moisturizing Factor Levels." *mBio* 9 (4): e01184-18. https://doi.org/10.1128/mBio.01184-18.

Finnegan, M., E. Duffy, and A. Morrin. 2022. "The Determination of Skin Surface pH via the Skin Volatile Emission Using Wearable Colorimetric Sensors." *Sensing and Bio-Sensing Research* 35 (February): 100473. https://doi.org/10.1016/j.sbsr.2022.100473.

Fleury, Orla M., Maeve A. McAleer, Cécile Feuillie, Cécile Formosa-Dague, Emily Sansevere, Désirée E. Bennett, Aisling M. Towell, et al. 2017. "Clumping Factor B Promotes Adherence of Staphylococcus Aureus to Corneocytes in Atopic Dermatitis." *Infection and Immunity* 85 (6): e00994-16. https://doi.org/10.1128/IAI.00994-16.

Flohr, C., D. Pascoe, and H. C. Williams. 2005. "Atopic Dermatitis and the 'Hygiene Hypothesis': Too Clean to Be True?" *The British Journal of Dermatology* 152 (2): 202–16. https://doi.org/10.1111/j.1365-2133.2004.06436.x.

Flowers, Laurice, and Elizabeth A. Grice. 2020. "The Skin Microbiota: Balancing Risk and Reward." *Cell Host & Microbe* 28 (2): 190–200. https://doi.org/10.1016/j.chom.2020.06.017.

Fluhr, Joachim W., Martin J. Behne, Barbara E. Brown, David G. Moskowitz, Clare Selden, Man Mao-Qiang, Theodora M. Mauro, Peter M. Elias, and Kenneth R. Feingold. 2004. "Stratum Corneum Acidification in Neonatal Skin: Secretory Phospholipase A2 and the Sodium/Hydrogen Antiporter-1 Acidify Neonatal Rat Stratum Corneum." *The Journal of Investigative Dermatology* 122 (2): 320–29. https://doi.org/10.1046/j.0022-202X.2003.00204.x.

Furue, Masutaka. 2020. "Regulation of Filaggrin, Loricrin, and Involucrin by IL-4, IL-13, IL-17A, IL-22, AHR, and NRF2: Pathogenic Implications in Atopic Dermatitis." *International Journal of Molecular Sciences* 21 (15): 5382. https://doi.org/10.3390/ijms21155382.

Fyhrquist, Nanna, Gareth Muirhead, Stefanie Prast-Nielsen, Marine Jeanmougin, Peter Olah, Tiina Skoog, Gerome Jules-Clement, et al. 2019. "Microbe-Host Interplay in Atopic Dermatitis and Psoriasis." *Nature Communications* 10 (1): 4703. https://doi.org/10.1038/s41467-019-12253-y.

Gallo, Richard L., and Teruaki Nakatsuji. 2011. "Microbial Symbiosis with the Innate Immune Defense System of the Skin." *The Journal of Investigative Dermatology* 131 (10): 1974–80. https://doi.org/10.1038/jid.2011.182.

Gao, Zhan, Guillermo I. Perez-Perez, Yu Chen, and Martin J. Blaser. 2010. "Quantitation of Major Human Cutaneous Bacterial and Fungal Populations." *Journal of Clinical Microbiology* 48 (10): 3575–81. https://doi.org/10.1128/jcm.00597-10.

Gilles, Stefanie, Cezmi Akdis, Roger Lauener, Peter Schmid-Grendelmeier, Thomas Bieber, Georg Schäppi, and Claudia Traidl-Hoffmann. 2018. "The Role of Environmental Factors in Allergy: A Critical Reappraisal." *Experimental Dermatology* 27 (11): 1193–1200. https://doi.org/10.1111/exd.13769.

Godlewska, Urszula, Piotr Brzoza, Kamila Kwiecień, Mateusz Kwitniewski, and Joanna Cichy. 2020. "Metagenomic Studies in Inflammatory Skin Diseases." *Current Microbiology* 77 (11): 3201–12. https://doi.org/10.1007/s00284-020-02163-4.

Goswami, Aranyak, Frank R. Wendt, Gita A. Pathak, Daniel S. Tylee, Flavio De Angelis, Antonella De Lillo, and Renato Polimanti. 2021. "Role of Microbes in the Pathogenesis of Neuropsychiatric Disorders." *Frontiers in Neuroendocrinology* 62 (July): 100917. https://doi.org/10.1016/j.yfrne.2021.100917.

Gour, Naina, and Stephane Lajoie. 2016. "Epithelial Cell Regulation of Allergic Diseases." *Current Allergy and Asthma Reports* 16 (9): 65. https://doi.org/10.1007/s11882-016-0640-7.

Grice, Elizabeth A., Heidi H. Kong, Sean Conlan, Clayton B. Deming, Joie Davis, Alice C. Young, Gerard G. Bouffard, et al. 2009. "Topographical and Temporal Diversity of the Human Skin Microbiome." *Science (New York, N.Y.)* 324 (5931): 1190–92. https://doi.org/10.1126/science.1171700.

Haller, Herbert Leopold, Frank Sander, Daniel Popp, Matthias Rapp, Bernd Hartmann, Mehmet Demircan, Sebastian Philipp Nischwitz, and Lars Peter Kamolz. 2021. "Oxygen, pH, Lactate, and Metabolism—How Old Knowledge and New Insights Might Be Combined for New Wound Treatment." *Medicina* 57 (11): 1190. https://doi.org/10.3390/medicina57111190.

Hanifin, Jon M., Wenelia Baghoomian, Erin Grinich, Yael A. Leshem, Michael Jacobson, and Eric Lawrence Simpson. 2022. "The Eczema Area and Severity Index—A Practical Guide." *Dermatitis* 33 (3): 187–92. https://doi.org/10.1097/DER.0000000000000895.

Hanssen, Anne-Merethe, Bert Kindlund, Niels Christian Stenklev, Anne-Sofie Furberg, Silje Fismen, Renate Slind Olsen, Mona Johannessen, and Johanna Ulrica Ericson Sollid. 2017. "Localization of Staphylococcus Aureus in Tissue from the Nasal Vestibule in Healthy Carriers." *BMC Microbiology* 17 (1): 89. https://doi.org/10.1186/s12866-017-0997-3.

Harris, L. G., S. J. Foster, and R. G. Richards. 2002. "An Introduction to Staphylococcus Aureus, and Techniques for Identifying and Quantifying S. Aureus Adhesins in Relation to Adhesion to Biomaterials: Review." *European Cells & Materials* 4 (December): 39–60. https://doi.org/10.22203/ecm.v004a04.

Hedderson, Monique M., Maryam M. Asgari, Fei Xu, Charles P. Quesenberry, Sneha Sridhar, Jamie Geier, and Adina R. Lemeshow. 2023. "Rates of Malignancies among Patients with Moderate to Severe Atopic Dermatitis: A Retrospective Cohort Study." *BMJ Open* 13 (3): e071172. https://doi.org/10.1136/bmjopen-2022-071172.

Henderson, John, Kate Northstone, Simon P. Lee, Haihui Liao, Yiwei Zhao, Marcus Pembrey, Somnath Mukhopadhyay, et al. 2008. "The Burden of Disease Associated with Filaggrin Mutations: A Population-Based, Longitudinal Birth Cohort Study." *Journal of Allergy and Clinical Immunology* 121 (4): 872-877.e9. https://doi.org/10.1016/j.jaci.2008.01.026.

Herrmann, Klaus M., and Lisa M. Weaver. 1999. “THE SHIKIMATE PATHWAY.” *Annual Review of Plant Physiology and Plant Molecular Biology* 50 (June): 473–503. https://doi.org/10.1146/annurev.arplant.50.1.473.

Hill, David A., and Jonathan M. Spergel. 2018. “The Atopic March: Critical Evidence and Clinical Relevance.” *Annals of Allergy, Asthma & Immunology : Official Publication of the American College of Allergy, Asthma, & Immunology* 120 (2): 131–37. https://doi.org/10.1016/j.anai.2017.10.037.

Hirayama, Daisuke, Tomoya Iida, and Hiroshi Nakase. 2017. “The Phagocytic Function of Macrophage-Enforcing Innate Immunity and Tissue Homeostasis.” *International Journal of Molecular Sciences* 19 (1): 92. https://doi.org/10.3390/ijms19010092.

Ho, Allen W., and Thomas S. Kupper. 2019. “T Cells and the Skin: From Protective Immunity to Inflammatory Skin Disorders.” *Nature Reviews. Immunology* 19 (8): 490–502. https://doi.org/10.1038/s41577-019-0162-3.

Hong, Hyo-Bong, In-Hyun Nam, Kumarasamy Murugesan, Young-Mo Kim, and Yoon-Seok Chang. 2004. “Biodegradation of Dibenzo-p-Dioxin, Dibenzofuran, and Chlorodibenzo-p-Dioxins by Pseudomonas Veronii PH-03.” *Biodegradation* 15 (5): 303–13. https://doi.org/10.1023/b:biod.0000042185.04905.0d.

Hooton, Kevin, Wei Han, and Liang Li. 2016. “Comprehensive and Quantitative Profiling of the Human Sweat Submetabolome Using High-Performance Chemical Isotope Labeling LC–MS.” Research-article. American Chemical Society. World. July 8, 2016. https://doi.org/10.1021/acs.analchem.6b01930.

Hrestak, Dora, Mario Matijašić, Hana Čipčić Paljetak, Daniela Ledić Drvar, Suzana Ljubojević Hadžavdić, and Mihaela Perić. 2022. “Skin Microbiota in Atopic Dermatitis.” *International Journal of Molecular Sciences* 23 (7): 3503. https://doi.org/10.3390/ijms23073503.

Hu, C., L. Duijts, N.S. Erler, N.J. Elbert, C. Piketty, V. Bourdès, S. Blanchet-Réthoré, et al. 2019. “Most Associations of Early-life Environmental Exposures and Genetic Risk Factors Poorly Differentiate between Eczema Phenotypes: The Generation R Study.” *The British Journal of Dermatology* 181 (6): 1190–97. https://doi.org/10.1111/bjd.17879.

Hu, Peng, Mengyao Wang, Hu Gao, Ai Zheng, Jinhui Li, Dezhi Mu, and Jiyu Tong. 2021. “The Role of Helper T Cells in Psoriasis.” *Frontiers in Immunology* 12. https://www.frontiersin.org/articles/10.3389/fimmu.2021.788940.

Hülpüsch, Claudia. 2021. “Value and Pitfalls of Skin Microbiome Research in Atopic Diseases Investigation of the Bacteria-Microenvironment Interaction.” Technischen Universität München.

Hülpüsch, Claudia, Karolina Tremmel, Gertrud Hammel, Madhumita Bhattacharyya, Amedeo de Tomassi, Thomas Nussbaumer, Avidan U. Neumann, Matthias Reiger, and Claudia Traidl-Hoffmann. 2020a. “Skin pH–Dependent Staphylococcus Aureus Abundance as Predictor for Increasing Atopic Dermatitis Severity.” *Allergy* 75 (11): 2888–98. https://doi.org/10.1111/all.14461.

———. 2020b. “Skin pH-Dependent Staphylococcus Aureus Abundance as Predictor for Increasing Atopic Dermatitis Severity.” *Allergy*, June. https://doi.org/10.1111/all.14461.

Hülpüsch, Claudia, Andreas B. Weins, Claudia Traidl-Hoffmann, and Matthias Reiger. 2021. “A New Era of Atopic Eczema Research: Advances and Highlights.” *Allergy* 76 (11): 3408–21. https://doi.org/10.1111/all.15058.

Ilic, Dusko, James M. Bollinger, Michael Gelb, and Theodora M. Mauro. 2014. “sPLA2 and the Epidermal Barrier.” *Biochimica Et Biophysica Acta* 1841 (3): 416–21. https://doi.org/10.1016/j.bbalip.2013.11.002.

Ilves, Liis, Aigar Ottas, Bret Kaldvee, Kristi Abram, Ursel Soomets, Mihkel Zilmer, Viljar Jaks, and Külli Kingo. 2021. "Metabolomic Analysis of Skin Biopsies from Patients with Atopic Dermatitis Reveals Hallmarks of Inflammation, Disrupted Barrier Function and Oxidative Stress." *Acta Dermato-Venereologica* 101 (2): adv00407. https://doi.org/10.2340/00015555-3766.

Iwamoto, Kazumasa, Masaya Moriwaki, Ryu Miyake, and Michihiro Hide. 2019. "Staphylococcus Aureus in Atopic Dermatitis: Strain-Specific Cell Wall Proteins and Skin Immunity." *Allergology International: Official Journal of the Japanese Society of Allergology* 68 (3): 309–15. https://doi.org/10.1016/j.alit.2019.02.006.

Iwamoto, Kazumasa, Masaya Moriwaki, Yoshie Niitsu, Masachika Saino, Shunsuke Takahagi, Junzo Hisatsune, Motoyuki Sugai, and Michihiro Hide. 2017. "Staphylococcus Aureus from Atopic Dermatitis Skin Alters Cytokine Production Triggered by Monocyte-Derived Langerhans Cell." *Journal of Dermatological Science* 88 (3): 271–79. https://doi.org/10.1016/j.jdermsci.2017.08.001.

Jang, Hyosun, Akira Matsuda, Kyungsook Jung, Kaoru Karasawa, Kenshiro Matsuda, Kumiko Oida, Saori Ishizaka, et al. 2016. "Skin pH Is the Master Switch of Kallikrein 5-Mediated Skin Barrier Destruction in a Murine Atopic Dermatitis Model." *The Journal of Investigative Dermatology* 136 (1): 127–35. https://doi.org/10.1038/JID.2015.363.

Jaworski, T. J., M. S. Sardessai, M. Aravagiri, G. Lin, Y. Y. Shi, E. M. Hawes, J. W. Hubbard, G. McKay, and K. K. Midha. 1993. "Synthesis of the N-Oxides of Phenothiazine Antipsychotic Agents." *Journal of Pharmaceutical Sciences* 82 (3): 330–33. https://doi.org/10.1002/jps.2600820323.

Jinnestål, Camilla Ling, Emma Belfrage, Ove Bäck, Artur Schmidtchen, and Andreas Sonesson. 2014. "Skin Barrier Impairment Correlates with Cutaneous Staphylococcus Aureus Colonization and Sensitization to Skin-Associated Microbial Antigens in Adult Patients with Atopic Dermatitis." *International Journal of Dermatology* 53 (1): 27–33. https://doi.org/10.1111/ijd.12198.

Jung, Eun Sung, Jong Il Park, Hyunjoon Park, Wilhelm Holzapfel, Jae Sung Hwang, and Choong Hwan Lee. 2019. "Seven-Day Green Tea Supplementation Revamps Gut Microbiome and Caecum/Skin Metabolome in Mice from Stress." *Scientific Reports* 9 (1): 18418. https://doi.org/10.1038/s41598-019-54808-5.

Kaplan, Allen, Mark Lebwohl, Ana M. Giménez-Arnau, Michihiro Hide, April W. Armstrong, and Marcus Maurer. 2023. "Chronic Spontaneous Urticaria: Focus on Pathophysiology to Unlock Treatment Advances." *Allergy* 78 (2): 389–401. https://doi.org/10.1111/all.15603.

Karlenius, Therese Christina, and Kathryn Fay Tonissen. 2010. "Thioredoxin and Cancer: A Role for Thioredoxin in All States of Tumor Oxygenation." *Cancers* 2 (2): 209–32. https://doi.org/10.3390/cancers2020209.

Katsarou, Spyridoula, Michael Makris, Efstratios Vakirlis, and Stamatios Gregoriou. 2023. "The Role of Tight Junctions in Atopic Dermatitis: A Systematic Review." *Journal of Clinical Medicine* 12 (4): 1538. https://doi.org/10.3390/jcm12041538.

Kim, Byung Eui, Elena Goleva, Peter S. Kim, Kathryn Norquest, Caroline Bronchick, Patricia Taylor, and Donald Y. M. Leung. 2019. "Side-by-Side Comparison of Skin Biopsies and Skin Tape Stripping Highlights Abnormal Stratum Corneum in Atopic Dermatitis." *The Journal of Investigative Dermatology* 139 (11): 2387-2389.e1. https://doi.org/10.1016/j.jid.2019.03.1160.

Kim, Byung Eui, and Donald Y.M. Leung. 2018. "Significance of Skin Barrier Dysfunction in Atopic Dermatitis." *Allergy, Asthma & Immunology Research* 10 (3): 207–15. https://doi.org/10.4168/aair.2018.10.3.207.

Kino-oka, Masahiro, Yuka Agatahama, Yuki Haga, Masukazu Inoie, and Masahito Taya. 2005. "Long-Term Subculture of Human Keratinocytes under an Anoxic Condition." *Journal of Bioscience and Bioengineering* 100 (1): 119–22. https://doi.org/10.1263/jbb.100.119.

Kobayashi, Tetsuro, Martin Glatz, Keisuke Horiuchi, Hiroshi Kawasaki, Haruhiko Akiyama, Daniel H. Kaplan, Heidi H. Kong, Masayuki Amagai, and Keisuke Nagao. 2015. "Dysbiosis and Staphylococcus Aureus Colonization Drives Inflammation in Atopic Dermatitis." *Immunity* 42 (4): 756–66. https://doi.org/10.1016/j.immuni.2015.03.014.

Kobayashi, Tetsuro, and Ichiro Imanishi. 2021. "Epithelial-Immune Crosstalk with the Skin Microbiota in Homeostasis and Atopic Dermatitis - a Mini Review." *Veterinary Dermatology* 32 (6): 533-e147. https://doi.org/10.1111/vde.13007.

Kondělková, Katerina, Doris Vokurková, Jan Krejsek, Lenka Borská, Zdeněk Fiala, and Andrýs Ctirad. 2010. "Regulatory T Cells (TREG) and Their Roles in Immune System with Respect to Immunopathological Disorders." *Acta Medica (Hradec Kralove)* 53 (2): 73–77. https://doi.org/10.14712/18059694.2016.63.

Kong, Heidi H., Björn Andersson, Thomas Clavel, John E. Common, Scott A. Jackson, Nathan D. Olson, Julia A. Segre, and Claudia Traidl-Hoffmann. 2017. "Performing Skin Microbiome Research: A Method to the Madness." *The Journal of Investigative Dermatology* 137 (3): 561–68. https://doi.org/10.1016/j.jid.2016.10.033.

Kool, Jeroen, Léon Reubsaet, Feikje Wesseldijk, Raquel T. Maravilha, Martijn W. Pinkse, Clive S. D'Santos, Jacobus J. van Hilten, Freek J. Zijlstra, and Albert J. R. Heck. 2007. "Suction Blister Fluid as Potential Body Fluid for Biomarker Proteins." *Proteomics* 7 (20): 3638–50. https://doi.org/10.1002/pmic.200600938.

Koutny, Marek, and Linda Zaoralkova. 2005. "Miniaturized Kinetic Growth Inhibition Assay with Denitrifying Bacteria Paracoccus Denitrificans." *Chemosphere* 60 (1): 49–54. https://doi.org/10.1016/j.chemosphere.2005.01.007.

Kramer, Owen N., Mark A. Strom, Barry Ladizinski, and Peter A. Lio. 2017. "The History of Atopic Dermatitis." *Clinics in Dermatology* 35 (4): 344–48. https://doi.org/10.1016/j.clindermatol.2017.03.005.

Krieken, Danique A. van der, Gijs Rikken, Thomas H.A. Ederveen, Patrick A.M. Jansen, Diana Rodijk-Olthuis, Luca D. Meesters, Ivonne M.J.J. van Vlijmen-Willems, et al. 2023. "Gram-Positive Anaerobic Cocci Guard Skin Homeostasis by Regulating Host-Defense Mechanisms." *iScience* 26 (4): 106483. https://doi.org/10.1016/j.isci.2023.106483.

Kuehne, Andreas, Janosch Hildebrand, Joern Soehle, Horst Wenck, Lara Terstegen, Stefan Gallinat, Anja Knott, Marc Winnefeld, and Nicola Zamboni. 2017. "An Integrative Metabolomics and Transcriptomics Study to Identify Metabolic Alterations in Aged Skin of Humans in Vivo." *BMC Genomics* 18 (1): 169. https://doi.org/10.1186/s12864-017-3547-3.

Kwon, Jung Hyun, Eunjeong Kim, Moon-Hee Chang, Eun Ae Park, Yun-Chul Hong, Mina Ha, Hyesook Park, Yangho Kim, Choonghee Park, and Eun-Hee Ha. 2015. "Indoor Total Volatile Organic Compounds Exposure at 6 Months Followed by Atopic Dermatitis at 3 Years in Children." *Pediatric Allergy and Immunology: Official Publication of the European Society of Pediatric Allergy and Immunology* 26 (4): 352–58. https://doi.org/10.1111/pai.12393.

Ladda, Matthew A., and Charles W. Lynde. 2019. "The Father of Medicine on Dermatology." *Journal of Cutaneous Medicine and Surgery* 23 (2): 237. https://doi.org/10.1177/1203475418811358.

Lagkouvardos, Ilias, Sandra Fischer, Neeraj Kumar, and Thomas Clavel. 2017. "Rhea: A Transparent and Modular R Pipeline for Microbial Profiling Based on 16S rRNA Gene Amplicons." *PeerJ* 5 (January): e2836. https://doi.org/10.7717/peerj.2836.

Lambers, H., S. Piessens, A. Bloem, H. Pronk, and P. Finkel. 2006. "Natural Skin Surface pH Is on Average below 5, Which Is Beneficial for Its Resident Flora." *International Journal of Cosmetic Science* 28 (5): 359–70. https://doi.org/10.1111/j.1467-2494.2006.00344.x.

Lamret, Fabien, Jennifer Varin-Simon, Frédéric Velard, Christine Terryn, Céline Mongaret, Marius Colin, Sophie C. Gangloff, and Fany Reffuveille. 2021. "Staphylococcus Aureus Strain-Dependent Biofilm Formation in Bone-Like Environment." *Frontiers in Microbiology* 12. https://www.frontiersin.org/articles/10.3389/fmicb.2021.714994.

Ledala, Nagender, Bo Zhang, Javier Seravalli, Robert Powers, and Greg A. Somerville. 2014. "Influence of Iron and Aeration on Staphylococcus Aureus Growth, Metabolism, and Transcription." *Journal of Bacteriology* 196 (12): 2178–89. https://doi.org/10.1128/JB.01475-14.

Legat, Franz J. 2021. "Itch in Atopic Dermatitis – What Is New?" *Frontiers in Medicine* 8. https://www.frontiersin.org/articles/10.3389/fmed.2021.644760.

Lehmann, H.S., T. Heaton, D. Mallon, and P.G. Holt. 2004. "Staphylococcal Enterotoxin-B-Mediated Stimulation of Interleukin-13 Production as a Potential Aetiologic Factor in Eczema in Infants." *International Archives of Allergy and Immunology* 135 (4): 306–12. https://doi.org/10.1159/000082324.

Lemaire, Sandrine, Françoise Van Bambeke, Marie-Paule Mingeot-Leclercq, Youri Glupczynski, and Paul M. Tulkens. 2007. "Role of Acidic pH in the Susceptibility of Intraphagocytic Methicillin-Resistant Staphylococcus Aureus Strains to Meropenem and Cloxacillin." *Antimicrobial Agents and Chemotherapy* 51 (5): 1627–32. https://doi.org/10.1128/AAC.01192-06.

Leung, D. Y. M., J. M. Hanifin, D. M. Pariser, K. A. Barber, R. G. Langley, P. M. Schlievert, B. Abrams, and T. Hultsch. 2009. "Effects of Pimecrolimus Cream 1% in the Treatment of Patients with Atopic Dermatitis Who Demonstrate a Clinical Insensitivity to Topical Corticosteroids: A Randomized, Multicentre Vehicle-Controlled Trial." *The British Journal of Dermatology* 161 (2): 435–43. https://doi.org/10.1111/j.1365-2133.2009.09145.x.

Levin, Jacquelyn, Sheila Fallon Friedlander, and James Q. Del Rosso. 2013a. "Atopic Dermatitis and the Stratum Corneum: Part 1: The Role of Filaggrin in the Stratum Corneum Barrier and Atopic Skin." *The Journal of Clinical and Aesthetic Dermatology* 6 (10): 16–22.

———. 2013b. "Atopic Dermatitis and the Stratum Corneum: Part 1: The Role of Filaggrin in the Stratum Corneum Barrier and Atopic Skin." *The Journal of Clinical and Aesthetic Dermatology* 6 (10): 16–22.

Levin, Jacquelyn, and Howard Maibach. 2008. "Human Skin Buffering Capacity: An Overview." *Skin Research and Technology: Official Journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI)* 14 (2): 121–26. https://doi.org/10.1111/j.1600-0846.2007.00271.x.

Lintzeri, D.a., N. Karimian, U. Blume-Peytavi, and J. Kottner. 2022. "Epidermal Thickness in Healthy Humans: A Systematic Review and Meta-Analysis." *Journal of the European Academy of Dermatology and Venereology* 36 (8): 1191–1200. https://doi.org/10.1111/jdv.18123.

Liu, Runqiu, Cong Peng, Danrong Jing, Yangjian Xiao, Wu Zhu, Shuang Zhao, Jianglin Zhang, Xiang Chen, and Jie Li. 2022. "Lachnospira Is a Signature of Antihistamine Efficacy in Chronic Spontaneous Urticaria." *Experimental Dermatology* 31 (2): 242–47. https://doi.org/10.1111/exd.14460.

Lodhia, M. H., K. R. Bhatt, and V. S. Thaker. 2009. "Antibacterial Activity of Essential Oils from Palmarosa, Evening Primrose, Lavender and Tuberose." *Indian Journal of Pharmaceutical Sciences* 71 (2): 134–36. https://doi.org/10.4103/0250-474X.54278.

Luo, Chia-Hui, Alan Chuan-Ying Lai, and Ya-Jen Chang. 2023. "Butyrate Inhibits Staphylococcus Aureus-Aggravated Dermal IL-33 Expression and Skin Inflammation through Histone Deacetylase Inhibition." *Frontiers in Immunology* 14: 1114699. https://doi.org/10.3389/fimmu.2023.1114699.

Luschkova, Daria, Katharina Zeiser, Alika Ludwig, and Claudia Traidl-Hoffmann. 2021. "Atopic Eczema Is an Environmental Disease." *Allergologie Select* 5: 244–50. https://doi.org/10.5414/ALX02258E.

Maintz, Laura, Thomas Welchowski, Nadine Herrmann, Juliette Brauer, Anna Sophie Kläschen, Rolf Fimmers, Matthias Schmid, et al. 2021. "Machine Learning-Based Deep Phenotyping of Atopic Dermatitis: Severity-Associated Factors in Adolescent and Adult Patients." *JAMA Dermatology* 157 (12): 1414–24. https://doi.org/10.1001/jamadermatol.2021.3668.

Masiuk, Helena, Aleksandra Wcisłek, and Joanna Jursa-Kulesza. 2021. "Determination of Nasal Carriage and Skin Colonization, Antimicrobial Susceptibility and Genetic Relatedness of Staphylococcus Aureus Isolated from Patients with Atopic Dermatitis in Szczecin, Poland." *BMC Infectious Diseases* 21 (July): 701. https://doi.org/10.1186/s12879-021-06382-3.

Matard, Bruno, Jean Luc Donay, Matthieu Resche-Rigon, Anne Tristan, David Farhi, Clotilde Rousseau, Severine Mercier-Delarue, et al. 2020. "Folliculitis Decalvans Is Characterized by a Persistent, Abnormal Subepidermal Microbiota." *Experimental Dermatology* 29 (3): 295–98. https://doi.org/10.1111/exd.13916.

Mathias, Rasika A., Sameer Chavan, Kruthika R. Iyer, Nicholas M. Rafaels, Meher Boorgula, Joseph Potee, Jon M. Hanifin, et al. 2015. "Identifying Genetic Determinants of Atopic Dermatitis and Bacterial Colonization Using Whole Genome Sequencing." *Journal of Allergy and Clinical Immunology* 135 (2): AB391. https://doi.org/10.1016/j.jaci.2014.12.1903.

Matsui, K., and A. Nishikawa. 2005. "Percutaneous Application of Peptidoglycan from Staphylococcus Aureus Induces an Increase in Mast Cell Numbers in the Dermis of Mice." *Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology* 35 (3): 382–87. https://doi.org/10.1111/j.1365-2222.2005.02190.x.

Matsui, Takeshi, Kenichi Miyamoto, Akiharu Kubo, Hiroshi Kawasaki, Tamotsu Ebihara, Kazuya Hata, Shinya Tanahashi, et al. 2011. "SASPase Regulates Stratum Corneum Hydration through Profilaggrin-to-Filaggrin Processing." *EMBO Molecular Medicine* 3 (6): 320–33. https://doi.org/10.1002/emmm.201100140.

McCaw, Ivan H. 1944. "A Synopsis of the History of Dermatology." *The Ulster Medical Journal* 13 (2): 109–22.

Mena-Bravo, A., and M. D. Luque de Castro. 2014. "Sweat: A Sample with Limited Present Applications and Promising Future in Metabolomics." *Journal of Pharmaceutical and Biomedical Analysis* 90 (March): 139–47. https://doi.org/10.1016/j.jpba.2013.10.048.

Merad, Miriam, Florent Ginhoux, and Matthew Collin. 2008. "Origin, Homeostasis and Function of Langerhans Cells and Other Langerin-Expressing Dendritic Cells." *Nature Reviews. Immunology* 8 (12): 935–47. https://doi.org/10.1038/nri2455.

Mews, Judyta, Agata Tomaszewska, Jacek Siewiera, Sławomir Lewicki, Karolina Kuczborska, Agnieszka Lipińska-Opałka, and Bolesław Kalicki. 2021. "Effects of Hyperbaric Oxygen Therapy in Children with Severe Atopic Dermatitis." *Journal of Clinical Medicine* 10 (6): 1157. https://doi.org/10.3390/jcm10061157.

Miajlovic, Helen, Padraic G. Fallon, Alan D. Irvine, and Timothy J. Foster. 2010. "Effect of Filaggrin Breakdown Products on Growth of and Protein Expression by Staphylococcus Aureus." *The Journal of Allergy and Clinical Immunology* 126 (6): 1184-1190.e3. https://doi.org/10.1016/j.jaci.2010.09.015.

Mieremet, Arnout, Adela Vázquez García, Walter Boiten, Rianne van Dijk, Gert Gooris, Joke A. Bouwstra, and Abdoelwaheb El Ghalbzouri. 2019. "Human Skin Equivalents Cultured under Hypoxia Display Enhanced Epidermal Morphogenesis and Lipid Barrier Formation." *Scientific Reports* 9 (1): 7811. https://doi.org/10.1038/s41598-019-44204-4.

Mierlo, Minke M.F. van, Luba M. Pardo, Karin B. Fieten, Tim J. van den Broek, Frank H.J. Schuren, Michel van Geel, and Suzanne G.M.A. Pasmans. 2022. "The Skin and Nose Microbiome and Its Association with Filaggrin Gene Mutations in Pediatric Atopic Dermatitis." *Dermatology* 238 (5): 928–38. https://doi.org/10.1159/000520978.

Misra, Namita, Cécile Clavaud, Florent Guinot, Nasrine Bourokba, Stephanie Nouveau, Sakina Mezzache, Paul Palazzi, et al. 2021. "Multi-Omics Analysis to Decipher the Molecular Link between Chronic Exposure to Pollution and Human Skin Dysfunction." *Scientific Reports* 11 (1): 18302. https://doi.org/10.1038/s41598-021-97572-1.

Moosbrugger-Martinz, Verena, Corinne Leprince, Marie-Claire Méchin, Michel Simon, Stefan Blunder, Robert Gruber, and Sandrine Dubrac. 2022. "Revisiting the Roles of Filaggrin in Atopic Dermatitis." *International Journal of Molecular Sciences* 23 (10): 5318. https://doi.org/10.3390/ijms23105318.

Moreau, Magali, Sophie Seité, Luc Aguilar, Olivier Da Cruz, Julia Puech, Johan Frieling, and Ann'Laure Demessant. 2021. "Topical S. Aureus - Targeting Endolysin Significantly Improves Symptoms and QoL in Individuals With Atopic Dermatitis." *Journal of Drugs in Dermatology: JDD* 20 (12): 1323–28. https://doi.org/10.36849/jdd.6363.

Moriwaki, Masaya, Kazumasa Iwamoto, Yoshie Niitsu, Ayako Matsushima, Yuhki Yanase, Junzo Hisatsune, Motoyuki Sugai, and Michihiro Hide. 2019. "Staphylococcus Aureus from Atopic Dermatitis Skin Accumulates in the Lysosomes of Keratinocytes with Induction of IL-1α Secretion via TLR9." *Allergy* 74 (3): 560–71. https://doi.org/10.1111/all.13622.

Morton, James T., Clarisse Marotz, Alex Washburne, Justin Silverman, Livia S. Zaramela, Anna Edlund, Karsten Zengler, and Rob Knight. 2019. "Establishing Microbial Composition Measurement Standards with Reference Frames." *Nature Communications* 10 (1): 2719. https://doi.org/10.1038/s41467-019-10656-5.

Murphy, Elizabeth Carmel, and Inga-Maria Frick. 2013. "Gram-Positive Anaerobic Cocci--Commensals and Opportunistic Pathogens." *FEMS Microbiology Reviews* 37 (4): 520–53. https://doi.org/10.1111/1574-6976.12005.

Mutius, E von. 2010. "99th Dahlem Conference on Infection, Inflammation and Chronic Inflammatory Disorders: Farm Lifestyles and the Hygiene Hypothesis." *Clinical and Experimental Immunology* 160 (1): 130–35. https://doi.org/10.1111/j.1365-2249.2010.04138.x.

Nakatsuji, Teruaki, Samantha L. Brinton, Kellen J. Cavagnero, Alan M. O'Neill, Yang Chen, Tatsuya Dokoshi, Anna M. Butcher, et al. 2023. "Competition between Skin Antimicrobial Peptides and Commensal Bacteria in Type 2 Inflammation Enables Survival of S. Aureus." *Cell Reports* 42 (5): 112494. https://doi.org/10.1016/j.celrep.2023.112494.

Nakatsuji, Teruaki, Tiffany H. Chen, Saisindhu Narala, Kimberly A. Chun, Aimee M. Two, Tong Yun, Faiza Shafiq, et al. 2017. "Antimicrobials from Human Skin Commensal Bacteria Protect against Staphylococcus Aureus and Are Deficient in Atopic

Dermatitis." *Science Translational Medicine* 9 (378). https://doi.org/10.1126/scitranslmed.aah4680.

Nakatsuji, Teruaki, Tiffany H. Chen, Aimee M. Two, Kimberly A. Chun, Saisindhu Narala, Raif S. Geha, Tissa R. Hata, and Richard L. Gallo. 2016. "Staphylococcus Aureus Exploits Epidermal Barrier Defects in Atopic Dermatitis to Trigger Cytokine Expression." *The Journal of Investigative Dermatology* 136 (11): 2192–2200. https://doi.org/10.1016/j.jid.2016.05.127.

Nakatsuji, Teruaki, Hsin-I Chiang, Shangi B. Jiang, Harish Nagarajan, Karsten Zengler, and Richard L. Gallo. 2013a. "The Microbiome Extends to Subepidermal Compartments of Normal Skin." *Nature Communications* 4: 1431. https://doi.org/10.1038/ncomms2441.

Nakatsuji, Teruaki, Hsin-I. Chiang, Shangi B. Jiang, Harish Nagarajan, Karsten Zengler, and Richard L. Gallo. 2013b. "The Microbiome Extends to Subepidermal Compartments of Normal Skin." *Nature Communications* 4 (February): 1431.

Napolitano, Maddalena, Francesca di Vico, Angelo Ruggiero, Gabriella Fabbrocini, and Cataldo Patruno. 2023. "The Hidden Sentinel of the Skin: An Overview on the Role of Interleukin-13 in Atopic Dermatitis." *Frontiers in Medicine* 10 (April): 1165098. https://doi.org/10.3389/fmed.2023.1165098.

Natsuga, Ken, Sara Cipolat, and Fiona M. Watt. 2016. "Increased Bacterial Load and Expression of Antimicrobial Peptides in Skin of Barrier-Deficient Mice with Reduced Cancer Susceptibility." *The Journal of Investigative Dermatology* 136 (1): 99–106. https://doi.org/10.1038/JID.2015.383.

Nestle, Frank O., Paola Di Meglio, Jian-Zhong Qin, and Brian J. Nickoloff. 2009. "Skin Immune Sentinels in Health and Disease." *Nature Reviews. Immunology* 9 (10): 679–91. https://doi.org/10.1038/nri2622.

Ngo, Mai A., Nadezda N. Sinitsyna, Qin Qin, and Robert H. Rice. 2007. "Oxygen-Dependent Differentiation of Human Keratinocytes." *Journal of Investigative Dermatology* 127 (2): 354–61. https://doi.org/10.1038/sj.jid.5700522.

Nguyen, Alan V., and Athena M. Soulika. 2019. "The Dynamics of the Skin's Immune System." *International Journal of Molecular Sciences* 20 (8): 1811. https://doi.org/10.3390/ijms20081811.

Niebuhr, Margarete, Merle Gathmann, Helena Scharonow, Diana Mamerow, Susanne Mommert, Hari Balaji, and Thomas Werfel. 2011. "Staphylococcal Alpha-Toxin Is a Strong Inducer of Interleukin-17 in Humans." *Infection and Immunity* 79 (4): 1615–22. https://doi.org/10.1128/IAI.00958-10.

Niedzwiecki, MeganM., Pradnya Samant, Douglas I. Walker, ViLinh Tran, Dean P. Jones, Mark R. Prausnitz, and Gary W. Miller. 2018. "Human Suction Blister Fluid Composition Determined Using High-Resolution Metabolomics." *Analytical Chemistry* 90 (6): 3786–92. https://doi.org/10.1021/acs.analchem.7b04073.

Nielsen, M L, D Raahave, J G Stage, and T Justesen. 1975. "Anaerobic and Aerobic Skin Bacteria before and after Skin-Disinfection with Chlorhexidine: An Experimental Study in Volunteers." *Journal of Clinical Pathology* 28 (10): 793–97.

Nikam, Rutuja Vilas, M Gowtham, Pratiksha Sanjay More, and Anuja Sanjay Shinde. 2023. "Current and Emerging Prospects in the Psoriatic Treatment." *International Immunopharmacology* 120 (July): 110331. https://doi.org/10.1016/j.intimp.2023.110331.

Nobles, Christopher L., Sabrina I. Green, and Anthony W. Maresso. 2013. "A Product of Heme Catabolism Modulates Bacterial Function and Survival." *PLoS Pathogens* 9 (7): e1003507. https://doi.org/10.1371/journal.ppat.1003507.

Nostro, Antonia, Luigina Cellini, Mara Di Giulio, Manuela D'Arrigo, Andreana Marino, Anna Rita Blanco, Angelo Favaloro, Giuseppina Cutroneo, and Giuseppe Bisignano. 2012.

"Effect of Alkaline pH on Staphylococcal Biofilm Formation." *APMIS* 120 (9): 733–42. https://doi.org/10.1111/j.1600-0463.2012.02900.x.

Nowicka, Danuta, and Urszula Nawrot. 2019. "Contribution of Malassezia Spp. to the Development of Atopic Dermatitis." *Mycoses* 62 (7): 588–96. https://doi.org/10.1111/myc.12913.

Nutten, Sophie. 2015. "Atopic Dermatitis: Global Epidemiology and Risk Factors." *Annals of Nutrition and Metabolism* 66 (Suppl. 1): 8–16. https://doi.org/10.1159/000370220.

Nys, Kris, Hannelore Maes, Graciela Andrei, Robert Snoeck, Maria Garmyn, and Patrizia Agostinis. 2012. "Skin Mild Hypoxia Enhances Killing of UVB-Damaged Keratinocytes through Reactive Oxygen Species-Mediated Apoptosis Requiring Noxa and Bim." *Free Radical Biology & Medicine* 52 (March): 1111–20. https://doi.org/10.1016/j.freeradbiomed.2011.12.017.

Nys, Kris, Hannelore Maes, Aleksandra Maria Dudek, and Patrizia Agostinis. 2011. "Uncovering the Role of Hypoxia Inducible Factor-1α in Skin Carcinogenesis." *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer* 1816 (1): 1–12. https://doi.org/10.1016/j.bbcan.2011.02.001.

Ochlich, Désirée, Franziska Rademacher, Katharina Antonia Drerup, Regine Gläser, and Jürgen Harder. 2023. "The Influence of the Commensal Skin Bacterium Staphylococcus Epidermidis on the Epidermal Barrier and Inflammation: Implications for Atopic Dermatitis." *Experimental Dermatology* 32 (4): 555–61. https://doi.org/10.1111/exd.14727.

Odhiambo, Joseph A., Hywel C. Williams, Tadd O. Clayton, Colin F. Robertson, M. Innes Asher, and ISAAC Phase Three Study Group. 2009. "Global Variations in Prevalence of Eczema Symptoms in Children from ISAAC Phase Three." *The Journal of Allergy and Clinical Immunology* 124 (6): 1251-1258.e23. https://doi.org/10.1016/j.jaci.2009.10.009.

Ofenloch, Robert F., Marie Louise Schuttelaar, Åke Svensson, Magnus Bruze, Luigi Naldi, Simone Cazzaniga, Peter Elsner, Margarida Gonçalo, and Thomas L. Diepgen. 2019. "Socioeconomic Status and the Prevalence of Skin and Atopic Diseases in Five European Countries." *Acta Dermato-Venereologica* 99 (3): 309–14. https://doi.org/10.2340/00015555-3082.

Ogonowska, Patrycja, Yolanda Gilaberte, Wioletta Barańska-Rybak, and Joanna Nakonieczna. 2021. "Colonization With Staphylococcus Aureus in Atopic Dermatitis Patients: Attempts to Reveal the Unknown." *Frontiers in Microbiology* 11 (January): 567090. https://doi.org/10.3389/fmicb.2020.567090.

Oh, Julia, Alexandra F. Freeman, NISC Comparative Sequencing Program, Morgan Park, Robert Sokolic, Fabio Candotti, Steven M. Holland, Julia A. Segre, and Heidi H. Kong. 2013. "The Altered Landscape of the Human Skin Microbiome in Patients with Primary Immunodeficiencies." *Genome Research* 23 (12): 2103–14. https://doi.org/10.1101/gr.159467.113.

Olesen, Caroline Meyer, Christine Sofie Krohn Fuchs, Peter Alshede Philipsen, Merete Hædersdal, Tove Agner, and Maja-Lisa Clausen. 2019. "Advancement through Epidermis Using Tape Stripping Technique and Reflectance Confocal Microscopy." *Scientific Reports* 9 (August): 12217. https://doi.org/10.1038/s41598-019-48698-w.

Ong, Guang Han, Daisuke Ori, Takumi Kawasaki, and Taro Kawai. 2022. "Inhibition of Lipopolysaccharide-Induced Inflammatory Responses by 1'-Acetoxychavicol Acetate." *Genes to Cells: Devoted to Molecular & Cellular Mechanisms* 27 (7): 482–92. https://doi.org/10.1111/gtc.12943.

Ong, Peck Y., and Donald Y. M. Leung. 2010. “The Infectious Aspects of Atopic Dermatitis.” *Immunology and Allergy Clinics of North America* 30 (3): 309–21. https://doi.org/10.1016/j.iac.2010.05.001.

———. 2016. “Bacterial and Viral Infections in Atopic Dermatitis: A Comprehensive Review.” *Clinical Reviews in Allergy & Immunology* 51 (3): 329–37. https://doi.org/10.1007/s12016-016-8548-5.

Ong, Peck Y., Takaaki Ohtake, Corinne Brandt, Ian Strickland, Mark Boguniewicz, Tomas Ganz, Richard L. Gallo, and Donald Y. M. Leung. 2002. “Endogenous Antimicrobial Peptides and Skin Infections in Atopic Dermatitis.” *The New England Journal of Medicine* 347 (15): 1151–60. https://doi.org/10.1056/NEJMoa021481.

Onyango, Laura A., and Mousa M. Alreshidi. 2018. “Adaptive Metabolism in Staphylococci: Survival and Persistence in Environmental and Clinical Settings.” *Journal of Pathogens* 2018 (September): e1092632. https://doi.org/10.1155/2018/1092632.

Palmer, Colin N. A., Alan D. Irvine, Ana Terron-Kwiatkowski, Yiwei Zhao, Haihui Liao, Simon P. Lee, David R. Goudie, et al. 2006. “Common Loss-of-Function Variants of the Epidermal Barrier Protein Filaggrin Are a Major Predisposing Factor for Atopic Dermatitis.” *Nature Genetics* 38 (4): 441–46. https://doi.org/10.1038/ng1767.

Panther, David J., and Sharon E. Jacob. 2015. “The Importance of Acidification in Atopic Eczema: An Underexplored Avenue for Treatment.” *Journal of Clinical Medicine* 4 (5): 970–78. https://doi.org/10.3390/jcm4050970.

Park, Young Joon, and Heung Kyu Lee. 2017. “The Role of Skin and Orogenital Microbiota in Protective Immunity and Chronic Immune-Mediated Inflammatory Disease.” *Frontiers in Immunology* 8: 1955. https://doi.org/10.3389/fimmu.2017.01955.

Patra, Vijaykumar, Natalie Bordag, Yohann Clement, Harald Köfeler, Jean-Francois Nicolas, Marc Vocanson, Sophie Ayciriex, and Peter Wolf. 2023. “Ultraviolet Exposure Regulates Skin Metabolome Based on the Microbiome.” *Scientific Reports* 13 (1): 7207. https://doi.org/10.1038/s41598-023-34073-3.

Paus, Ralf, Theoharis C. Theoharides, and Petra Clara Arck. 2006. “Neuroimmunoendocrine Circuitry of the ‘Brain-Skin Connection.’” *Trends in Immunology* 27 (1): 32–39. https://doi.org/10.1016/j.it.2005.10.002.

Pham, Duy Le, Kyung-Min Lim, Kyung-Mi Joo, Hae-Sim Park, Donald Y. M. Leung, and Young-Min Ye. 2017. “Increased Cis-to-Trans Urocanic Acid Ratio in the Skin of Chronic Spontaneous Urticaria Patients.” *Scientific Reports* 7 (May): 1318. https://doi.org/10.1038/s41598-017-01487-9.

Pistone, Dario, Gabriele Meroni, Simona Panelli, Enza D’Auria, Miriam Acunzo, Ajay Ratan Pasala, Gian Vincenzo Zuccotti, Claudio Bandi, and Lorenzo Drago. 2021. “A Journey on the Skin Microbiome: Pitfalls and Opportunities.” *International Journal of Molecular Sciences* 22 (18): 9846. https://doi.org/10.3390/ijms22189846.

Proksch, Ehrhardt. 2018a. “pH in Nature, Humans and Skin.” *The Journal of Dermatology* 45 (9): 1044–52. https://doi.org/10.1111/1346-8138.14489.

———. 2018b. “pH in Nature, Humans and Skin.” *The Journal of Dermatology* 45 (9): 1044–52. https://doi.org/10.1111/1346-8138.14489.

Pucci, Neri, Enrico Lombardi, Elio Novembre, Silvia Farina, Roberto Bernardini, Elisabetta Rossi, Tania Favilli, and Alberto Vierucci. 2000. “Urinary Eosinophil Protein X and Serum Eosinophil Cationic Protein in Infants and Young Children with Atopic Dermatitis: Correlation with Disease Activity.” *Journal of Allergy and Clinical Immunology* 105 (2, Part 1): 353–57. https://doi.org/10.1016/S0091-6749(00)90087-3.

Quale, John M., Laura J. Mandel, Nora V. Bergasa, and Eugene W. Straus. 1988. “Clinical Significance and Pathogenesis of Hyperbilirubinemia Associated with Staphylococcus

Aureus Septicemia." *The American Journal of Medicine* 85 (5): 615–18. https://doi.org/10.1016/S0002-9343(88)80231-6.

Quan, Cheng, Xiao-Ying Chen, Xia Li, Feng Xue, Li-Hong Chen, Na Liu, Bo Wang, et al. 2020. "Psoriatic Lesions Are Characterized by Higher Bacterial Load and Imbalance between Cutibacterium and Corynebacterium." *Journal of the American Academy of Dermatology* 82 (4): 955–61. https://doi.org/10.1016/j.jaad.2019.06.024.

Quaranta, Maria, Bettina Knapp, Natalie Garzorz, Martina Mattii, Venu Pullabhatla, Davide Pennino, Christian Andres, et al. 2014. "Intraindividual Genome Expression Analysis Reveals a Specific Molecular Signature of Psoriasis and Eczema." *Science Translational Medicine* 6 (244): 244ra90. https://doi.org/10.1126/scitranslmed.3008946.

R Core Team. 2022. "R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria." 2022. https://www.R-project.org/.

Raharja, Antony, Satveer K. Mahil, and Jonathan N. Barker. 2021. "Psoriasis: A Brief Overview." *Clinical Medicine (London, England)* 21 (3): 170–73. https://doi.org/10.7861/clinmed.2021-0257.

Randhawa, Manpreet, Vineet Sangar, Samantha Tucker-Samaras, and Michael Southall. 2014. "Metabolic Signature of Sun Exposed Skin Suggests Catabolic Pathway Overweighs Anabolic Pathway." *PLOS ONE* 9 (3): e90367. https://doi.org/10.1371/journal.pone.0090367.

Rauer, Luise, Matthias Reiger, Madhumita Bhattacharyya, Patrick M. Brunner, James G. Krueger, Emma Guttman-Yassky, Claudia Traidl-Hoffmann, and Avidan U. Neumann. 2023. "Skin Microbiome and Its Association with Host Cofactors in Determining Atopic Dermatitis Severity." *Journal of the European Academy of Dermatology and Venereology: JEADV* 37 (4): 772–82. https://doi.org/10.1111/jdv.18776.

Reiger, Matthias, Claudia Traidl-Hoffmann, and Avidan U. Neumann. 2020. "The Skin Microbiome as a Clinical Biomarker in Atopic Eczema: Promises, Navigation, and Pitfalls." *Journal of Allergy and Clinical Immunology* 145 (1): 93–96. https://doi.org/10.1016/j.jaci.2019.11.004.

Rezvani, Hamid R., Nsrein Ali, Lars J. Nissen, Ghida Harfouche, Hubert de Verneuil, Alain Taïeb, and Frédéric Mazurier. 2011. "HIF-1α in Epidermis: Oxygen Sensing, Cutaneous Angiogenesis, Cancer, and Non-Cancer Disorders." *Journal of Investigative Dermatology* 131 (9): 1793–1805. https://doi.org/10.1038/jid.2011.141.

Rie, Menno A. de, Amber Y. Goedkoop, and Jan D. Bos. 2004. "Overview of Psoriasis." *Dermatologic Therapy* 17 (5): 341–49. https://doi.org/10.1111/j.1396-0296.2004.04037.x.

Rode, Tone Mari, Trond Møretrø, Solveig Langsrud, Oyvind Langsrud, Gjermund Vogt, and Askild Holck. 2010. "Responses of Staphylococcus Aureus Exposed to HCl and Organic Acid Stress." *Canadian Journal of Microbiology* 56 (9): 777–92. https://doi.org/10.1139/w10-057.

Roduit, Caroline, Remo Frei, Martin Depner, Anne M. Karvonen, Harald Renz, Charlotte Braun-Fahrländer, Elisabeth Schmausser-Hechfellner, et al. 2017. "Phenotypes of Atopic Dermatitis Depending on the Timing of Onset and Progression in Childhood." *JAMA Pediatrics* 171 (7): 655–62. https://doi.org/10.1001/jamapediatrics.2017.0556.

Roosterman, Dirk, Tobias Goerge, Stefan W. Schneider, Nigel W. Bunnett, and Martin Steinhoff. 2006. "Neuronal Control of Skin Function: The Skin as a Neuroimmunoendocrine Organ." *Physiological Reviews* 86 (4): 1309–79. https://doi.org/10.1152/physrev.00026.2005.

Roth, Harry L., and Robert R. Kierland. 1964. “The Natural History of Atopic Dermatitis: A 20-Year Follow-Up Study.” *Archives of Dermatology* 89 (2): 209–14. https://doi.org/10.1001/archderm.1964.01590260047008.

Rump, B., M. De Boer, R. Reis, M. Wassenberg, and J. Van Steenbergen. 2017. “Signs of Stigma and Poor Mental Health among Carriers of MRSA.” *The Journal of Hospital Infection* 95 (3): 268–74. https://doi.org/10.1016/j.jhin.2016.09.010.

Saito, Hirohisa. 2005. “Much Atopy about the Skin: Genome-Wide Molecular Analysis of Atopic Eczema.” *International Archives of Allergy and Immunology* 137 (4): 319–25. https://doi.org/10.1159/000086464.

Sakr, Adèle, Fabienne Brégeon, Jean-Louis Mège, Jean-Marc Rolain, and Olivier Blin. 2018. “Staphylococcus Aureus Nasal Colonization: An Update on Mechanisms, Epidemiology, Risk Factors, and Subsequent Infections.” *Frontiers in Microbiology* 9. https://www.frontiersin.org/articles/10.3389/fmicb.2018.02419.

Samant, Pradnya P., Megan M. Niedzwiecki, Nicholas Raviele, Vilinh Tran, Juan Mena-Lapaix, Douglas I. Walker, Eric I. Felner, Dean P. Jones, Gary W. Miller, and Mark R. Prausnitz. 2020. “Sampling Interstitial Fluid from Human Skin Using a Microneedle Patch.” *Science Translational Medicine*, November. https://doi.org/10.1126/scitranslmed.aaw0285.

Sandby-Møller, Jane, Thomas Poulsen, and Hans Christian Wulf. 2003. “Epidermal Thickness at Different Body Sites: Relationship to Age, Gender, Pigmentation, Blood Content, Skin Type and Smoking Habits.” *Acta Dermato-Venereologica* 83 (6): 410–13. https://doi.org/10.1080/00015550310015419.

Sandilands, Aileen, Calum Sutherland, Alan D. Irvine, and W. H. Irwin McLean. 2009. “Filaggrin in the Frontline: Role in Skin Barrier Function and Disease.” *Journal of Cell Science* 122 (Pt 9): 1285–94. https://doi.org/10.1242/jcs.033969.

Sato, K., W. H. Kang, K. Saga, and K. T. Sato. 1989. “Biology of Sweat Glands and Their Disorders. I. Normal Sweat Gland Function.” *Journal of the American Academy of Dermatology* 20 (4): 537–63. https://doi.org/10.1016/s0190-9622(89)70063-3.

Schäfer, L., and K. Kragballe. 1991. “Abnormalities in Epidermal Lipid Metabolism in Patients with Atopic Dermatitis.” *The Journal of Investigative Dermatology* 96 (1): 10–15. https://doi.org/10.1111/1523-1747.ep12514648.

Scharschmidt, Tiffany C., Kimberly S. Vasquez, Hong-An Truong, Sofia V. Gearty, Mariela L. Pauli, Audrey Nosbaum, Iris K. Gratz, et al. 2015. “A Wave of Regulatory T Cells into Neonatal Skin Mediates Tolerance to Commensal Microbes.” *Immunity* 43 (5): 1011–21. https://doi.org/10.1016/j.immuni.2015.10.016.

Schittek, B., R. Hipfel, B. Sauer, J. Bauer, H. Kalbacher, S. Stevanovic, M. Schirle, et al. 2001. “Dermcidin: A Novel Human Antibiotic Peptide Secreted by Sweat Glands.” *Nature Immunology* 2 (12): 1133–37. https://doi.org/10.1038/ni732.

Schlievert, Patrick M., Laura C. Case, Kristi L. Strandberg, Bea B. Abrams, and Donald Y. M. Leung. 2008. “Superantigen Profile of Staphylococcus Aureus Isolates from Patients with Steroid-Resistant Atopic Dermatitis.” *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* 46 (10): 1562–67. https://doi.org/10.1086/586746.

Schlievert, Patrick M., Kristi L. Strandberg, Ying-Chi Lin, Marnie L. Peterson, and Donald Y. M. Leung. 2010. “Secreted Virulence Factor Comparison between Methicillin-Resistant and Methicillin-Sensitive Staphylococcus Aureus, and Its Relevance to Atopic Dermatitis.” *The Journal of Allergy and Clinical Immunology* 125 (1): 39–49. https://doi.org/10.1016/j.jaci.2009.10.039.

Schmid-Wendtner, M.-H., and H. C. Korting. 2006. “The pH of the Skin Surface and Its Impact on the Barrier Function.” *Skin Pharmacology and Physiology* 19 (6): 296–302. https://doi.org/10.1159/000094670.

Schonmann, Yochai, Kathryn E. Mansfield, Joseph F. Hayes, Katrina Abuabara, Amanda Roberts, Liam Smeeth, and Sinéad M. Langan. 2020. “Atopic Eczema in Adulthood and Risk of Depression and Anxiety: A Population-Based Cohort Study.” *The Journal of Allergy and Clinical Immunology. in Practice* 8 (1): 248-257.e16. https://doi.org/10.1016/j.jaip.2019.08.030.

Schreml, Stephan, Robert J. Meier, Otto S. Wolfbeis, Michael Landthaler, Rolf-Markus Szeimies, and Philipp Babilas. 2011. “2D Luminescence Imaging of pH in Vivo.” *Proceedings of the National Academy of Sciences of the United States of America* 108 (6): 2432–37. https://doi.org/10.1073/pnas.1006945108.

Schwarz, Agatha, Anika Bruhs, and Thomas Schwarz. 2017. “The Short-Chain Fatty Acid Sodium Butyrate Functions as a Regulator of the Skin Immune System.” *The Journal of Investigative Dermatology* 137 (4): 855–64. https://doi.org/10.1016/j.jid.2016.11.014.

Serpi, Michaela, Valentina Ferrari, and Fabrizio Pertusati. 2016. “Nucleoside Derived Antibiotics to Fight Microbial Drug Resistance: New Utilities for an Established Class of Drugs?” *Journal of Medicinal Chemistry* 59 (23): 10343–82. https://doi.org/10.1021/acs.jmedchem.6b00325.

Shen, Wei, Wenqing Li, Julie A. Hixon, Nicolas Bouladoux, Yasmine Belkaid, Amiran Dzutzev, and Scott K. Durum. 2014. “Adaptive Immunity to Murine Skin Commensals.” *Proceedings of the National Academy of Sciences of the United States of America* 111 (29): E2977-2986. https://doi.org/10.1073/pnas.1401820111.

Sher, Lindsay G., Jongwha Chang, Isha B. Patel, Rajesh Balkrishnan, and Alan B. Fleischer. 2012. “Relieving the Pruritus of Atopic Dermatitis: A Meta-Analysis.” *Acta Dermato-Venereologica* 92 (5): 455–61. https://doi.org/10.2340/00015555-1360.

Shu, Muya, Yanhan Wang, Jinghua Yu, Sherwin Kuo, Alvin Coda, Yong Jiang, Richard L. Gallo, and Chun-Ming Huang. 2013. “Fermentation of Propionibacterium Acnes, a Commensal Bacterium in the Human Skin Microbiome, as Skin Probiotics against Methicillin-Resistant Staphylococcus Aureus.” *PLoS ONE* 8 (2): e55380. https://doi.org/10.1371/journal.pone.0055380.

Sillner, Nina, Alesia Walker, Eva-Maria Harrieder, Philippe Schmitt-Kopplin, and Michael Witting. 2019. “Development and Application of a HILIC UHPLC-MS Method for Polar Fecal Metabolome Profiling.” *Journal of Chromatography B* 1109 (March): 142–48. https://doi.org/10.1016/j.jchromb.2019.01.016.

Silverberg, Jonathan I., Sebastien Barbarot, Abhijit Gadkari, Eric L. Simpson, Stephan Weidinger, Paola Mina-Osorio, Ana B. Rossi, et al. 2021. “Atopic Dermatitis in the Pediatric Population: A Cross-Sectional, International Epidemiologic Study.” *Annals of Allergy, Asthma & Immunology: Official Publication of the American College of Allergy, Asthma, & Immunology* 126 (4): 417-428.e2. https://doi.org/10.1016/j.anai.2020.12.020.

Simpson, Cory L., Dipal M. Patel, and Kathleen J. Green. 2011. “Deconstructing the Skin: Cytoarchitectural Determinants of Epidermal Morphogenesis.” *Nature Reviews. Molecular Cell Biology* 12 (9): 565–80. https://doi.org/10.1038/nrm3175.

Simpson, Eric L., Miguel Villarreal, Brett Jepson, Nick Rafaels, Gloria David, Jon Hanifin, Patricia Taylor, et al. 2018. “Patients with Atopic Dermatitis Colonized with *Staphylococcus Aureus* Have a Distinct Phenotype and Endotype.” *Journal of Investigative Dermatology* 138 (10): 2224–33. https://doi.org/10.1016/j.jid.2018.03.1517.

Sinclair, Eleanor, Drupad K. Trivedi, Depanjan Sarkar, Caitlin Walton-Doyle, Joy Milne, Tilo Kunath, Anouk M. Rijs, et al. 2021. "Metabolomics of Sebum Reveals Lipid Dysregulation in Parkinson's Disease." *Nature Communications* 12 (1): 1592. https://doi.org/10.1038/s41467-021-21669-4.

Skowron, Krzysztof, Justyna Bauza-Kaszewska, Zuzanna Kraszewska, Natalia Wiktorczyk-Kapischke, Katarzyna Grudlewska-Buda, Joanna Kwiecińska-Piróg, Ewa Wałecka-Zacharska, Laura Radtke, and Eugenia Gospodarek-Komkowska. 2021. "Human Skin Microbiome: Impact of Intrinsic and Extrinsic Factors on Skin Microbiota." *Microorganisms* 9 (3): 543. https://doi.org/10.3390/microorganisms9030543.

Smeden, J. van, M. Janssens, G. S. Gooris, and J. A. Bouwstra. 2014. "The Important Role of Stratum Corneum Lipids for the Cutaneous Barrier Function." *Biochimica Et Biophysica Acta* 1841 (3): 295–313. https://doi.org/10.1016/j.bbalip.2013.11.006.

Smeden, Jeroen van, Walter A. Boiten, Thomas Hankemeier, Robert Rissmann, Joke A. Bouwstra, and Rob J. Vreeken. 2014. "Combined LC/MS-Platform for Analysis of All Major Stratum Corneum Lipids, and the Profiling of Skin Substitutes." *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* 1841 (1): 70–79. https://doi.org/10.1016/j.bbalip.2013.10.002.

Sølberg, Julie, Nina Ulrich, Dorrit Krustrup, Malin Ahlström, Jacob Thyssen, Torkil Menné, Charlotte Bonefeld, Anne-Sofie Gadsbøll, Eva Balslev, and Jeanne Johansen. 2018. "Skin Tape Stripping: Which Layers of the Epidermis Are Removed?" *Contact Dermatitis* 80 (December). https://doi.org/10.1111/cod.13199.

Somerville, Greg A., Michael S. Chaussee, Carrie I. Morgan, J. Ross Fitzgerald, David W. Dorward, Lawrence J. Reitzer, and James M. Musser. 2002. "Staphylococcus Aureus Aconitase Inactivation Unexpectedly Inhibits Post-Exponential-Phase Growth and Enhances Stationary-Phase Survival." *Infection and Immunity* 70 (11): 6373–82. https://doi.org/10.1128/IAI.70.11.6373-6382.2002.

Somerville, Greg A., Battouli Saïd-Salim, Jaala M. Wickman, Sandra J. Raffel, Barry N. Kreiswirth, and James M. Musser. 2003. "Correlation of Acetate Catabolism and Growth Yield in Staphylococcus Aureus: Implications for Host-Pathogen Interactions." *Infection and Immunity* 71 (8): 4724–32. https://doi.org/10.1128/IAI.71.8.4724-4732.2003.

Sparavigna, A., M. Setaro, and V. Gualandri. 1999. "Cutaneous pH in Children Affected by Atopic Dermatitis and in Healthy Children: A Multicenter Study." *Skin Research and Technology* 5 (4): 221–27. https://doi.org/10.1111/j.1600-0846.1999.tb00134.x.

Steiman, Cheryl A., Michael D. Evans, Kristine E. Lee, Michael R. Lasarev, Ronald E. Gangnon, Brent F. Olson, Kathrine L. Barnes, Casper G. Bendixsen, Christine M. Seroogy, and James E. Gern. 2020. "Patterns of Farm Exposure Are Associated with Reduced Incidence of Atopic Dermatitis in Early Life." *The Journal of Allergy and Clinical Immunology* 146 (6): 1379-1386.e6. https://doi.org/10.1016/j.jaci.2020.06.025.

Stein, Michelle M., Cara L. Hrusch, Justyna Gozdz, Catherine Igartua, Vadim Pivniouk, Sean E. Murray, Julie G. Ledford, et al. 2016. "Innate Immunity and Asthma Risk in Amish and Hutterite Farm Children." *The New England Journal of Medicine* 375 (5): 411–21. https://doi.org/10.1056/NEJMoa1508749.

Steinhoff, Martin, Sonja Ständer, Stephan Seeliger, John C. Ansel, Martin Schmelz, and Thomas Luger. 2003. "Modern Aspects of Cutaneous Neurogenic Inflammation." *Archives of Dermatology* 139 (11): 1479–88. https://doi.org/10.1001/archderm.139.11.1479.

Strachan, D. P. 1989. "Hay Fever, Hygiene, and Household Size." *BMJ : British Medical Journal* 299 (6710): 1259–60.

Sugiura, H., H. Ebise, T. Tazawa, K. Tanaka, Y. Sugiura, M. Uehara, K. Kikuchi, and T. Kimura. 2005. "Large-Scale DNA Microarray Analysis of Atopic Skin Lesions Shows Overexpression of an Epidermal Differentiation Gene Cluster in the Alternative Pathway and Lack of Protective Gene Expression in the Cornified Envelope." *The British Journal of Dermatology* 152 (1): 146–49. https://doi.org/10.1111/j.1365-2133.2005.06352.x.

Suhre, Karsten, and Philippe Schmitt-Kopplin. 2008. "MassTRIX: Mass Translator into Pathways." *Nucleic Acids Research* 36 (Web Server issue): W481-484. https://doi.org/10.1093/nar/gkn194.

Sumner, Lloyd W., Alexander Amberg, Dave Barrett, Michael H. Beale, Richard Beger, Clare A. Daykin, Teresa W.-M. Fan, et al. 2007. "Proposed Minimum Reporting Standards for Chemical Analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI)." *Metabolomics : Official Journal of the Metabolomic Society* 3 (3): 211–21. https://doi.org/10.1007/s11306-007-0082-2.

Swaney, Mary Hannah, and Lindsay R. Kalan. 2021. "Living in Your Skin: Microbes, Molecules, and Mechanisms." *Infection and Immunity* 89 (4): e00695-20. https://doi.org/10.1128/IAI.00695-20.

Tabata, N., H. Tagami, and A. M. Kligman. 1998. "A Twenty-Four-Hour Occlusive Exposure to 1% Sodium Lauryl Sulfate Induces a Unique Histopathologic Inflammatory Response in the Xerotic Skin of Atopic Dermatitis Patients." *Acta Dermato-Venereologica* 78 (4): 244–47. https://doi.org/10.1080/000155598441792.

Taïeb, A., D. Wallach, and G. Tilles. 2006. "The History of Atopic Eczema/Dermatitis." In *Handbook of Atopic Eczema*, edited by Johannes Ring, Bernhard Przybilla, and Thomas Ruzicka, 10–20. Berlin, Heidelberg: Springer. https://doi.org/10.1007/3-540-29856-8_2.

Taylor, Tracey A., and Chandrashekhar G. Unakal. 2023. "Staphylococcus Aureus Infection." In *StatPearls*. Treasure Island (FL): StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK441868/.

Thornley, B., J. Rathbone, C. E. Adams, and G. Awad. 2003. "Chlorpromazine versus Placebo for Schizophrenia." *The Cochrane Database of Systematic Reviews*, no. 2: CD000284. https://doi.org/10.1002/14651858.CD000284.

Totté, J. E. E., W. T. van der Feltz, M. Hennekam, A. van Belkum, E. J. van Zuuren, and S. G. M. A. Pasmans. 2016. "Prevalence and Odds of Staphylococcus Aureus Carriage in Atopic Dermatitis: A Systematic Review and Meta-Analysis." *The British Journal of Dermatology* 175 (4): 687–95. https://doi.org/10.1111/bjd.14566.

Traisaeng, Supitchaya, Deron Raymond Herr, Hsin-Jou Kao, Tsung-Hsien Chuang, and Chun-Ming Huang. 2019. "A Derivative of Butyric Acid, the Fermentation Metabolite of Staphylococcus Epidermidis, Inhibits the Growth of a Staphylococcus Aureus Strain Isolated from Atopic Dermatitis Patients." *Toxins* 11 (6): 311. https://doi.org/10.3390/toxins11060311.

Trivedi, DrupadK., Eleanor Sinclair, Yun Xu, Depanjan Sarkar, Caitlin Walton-Doyle, Camilla Liscio, Phine Banks, et al. 2019. "Discovery of Volatile Biomarkers of Parkinson's Disease from Sebum." *ACS Central Science* 5 (4): 599–606. https://doi.org/10.1021/acscentsci.8b00879.

Turkalj, Mirjana, Vlado Drkulec, Sadia Haider, Davor Plavec, Ivana Banić, Olga Malev, Damir Erceg, Ashley Woodcock, Boro Nogalo, and Adnan Custovic. 2020. "Association of Bacterial Load in Drinking Water and Allergic Diseases in Childhood." *Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology* 50 (6): 733–40. https://doi.org/10.1111/cea.13605.

Turner, N. G., C. Cullander, and R. H. Guy. 1998. “Determination of the pH Gradient across the Stratum Corneum.” *The Journal of Investigative Dermatology. Symposium Proceedings* 3 (2): 110–13. https://doi.org/10.1038/jidsymp.1998.23.

Uwaifo, A. O., D. A. Okorie, and E. A. Bababunmi. 1979. “Mutagenicity of Chamuvaritin: A Benzyldihydrochalcone Isolated from a Medicinal Plant.” *Cancer Letters* 8 (1): 87–92. https://doi.org/10.1016/0304-3835(79)90027-2.

Větrovský, Tomáš, and Petr Baldrian. 2013. “The Variability of the 16S rRNA Gene in Bacterial Genomes and Its Consequences for Bacterial Community Analyses.” *PLoS ONE* 8 (2): e57923. https://doi.org/10.1371/journal.pone.0057923.

Viant, Mark R, Irwin J Kurland, Martin R Jones, and Warwick B Dunn. 2017. “How Close Are We to Complete Annotation of Metabolomes?” *Current Opinion in Chemical Biology*, Omics, 36 (February): 64–69. https://doi.org/10.1016/j.cbpa.2017.01.001.

Voss, Martin, Johanna Kotrba, Evelyn Gaffal, Konstantinos Katsoulis-Dimitriou, and Anne Dudeck. 2021. “Mast Cells in the Skin: Defenders of Integrity or Offenders in Inflammation?” *International Journal of Molecular Sciences* 22 (9): 4589. https://doi.org/10.3390/ijms22094589.

Wang, Xiaofan, Samantha Howe, Feilong Deng, and Jiangchao Zhao. 2021. “Current Applications of Absolute Bacterial Quantification in Microbiome Studies and Decision-Making Regarding Different Biological Questions.” *Microorganisms* 9 (9): 1797. https://doi.org/10.3390/microorganisms9091797.

Watanakunakorn, C., S. J. Chan, D. G. Demarco, and J. A. Palmer. 1987. “Staphylococcus Aureus Bacteremia: Significance of Hyperbilirubinemia.” *Scandinavian Journal of Infectious Diseases* 19 (2): 195–203. https://doi.org/10.3109/00365548709032398.

Weckel, Antonin, Miqdad O. Dhariwala, Kevin Ly, Victoria M. Tran, Oluwasunmisola T. Ojewumi, Julianne B. Riggs, Jeanmarie R. Gonzalez, et al. 2023. “Long-Term Tolerance to Skin Commensals Is Established Neonatally through a Specialized Dendritic Cell Subgroup.” *Immunity*, March, S1074-7613(23)00128-0. https://doi.org/10.1016/j.immuni.2023.03.008.

Weerakkody, Nimsha S., William M. Smith, Deirdre Mikkelsen, Jennifer Waanders, Graham Kerven, Nola Caffin, Gary A. Dykes, and Mark S. Turner. 2012. “Purified 1′acetoxychavicol Acetate (1′ACA) from Galangal Spice Affects Membrane Fatty Acid Composition and Triggers a Cell Envelope Stress Response in Staphylococcus Aureus.” *International Journal of Antimicrobial Agents* 39 (3): 269–71. https://doi.org/10.1016/j.ijantimicag.2011.11.010.

Weinrick, Brian, Paul M. Dunman, Fionnuala McAleese, Ellen Murphy, Steven J. Projan, Yuan Fang, and Richard P. Novick. 2004. “Effect of Mild Acid on Gene Expression in Staphylococcus Aureus.” *Journal of Bacteriology* 186 (24): 8407–23. https://doi.org/10.1128/JB.186.24.8407-8423.2004.

Werfel, Thomas, Jean-Pierre Allam, Tilo Biedermann, Kilian Eyerich, Stefanie Gilles, Emma Guttman-Yassky, Wolfram Hoetzenecker, et al. 2016. “Cellular and Molecular Immunologic Mechanisms in Patients with Atopic Dermatitis.” *Journal of Allergy and Clinical Immunology* 138 (2): 336–49. https://doi.org/10.1016/j.jaci.2016.06.010.

Whipps, John M., Karen Lewis, and R. C. Cooke. 1988. “Mycoparasitism and Plant Disease Control.” *Fungi in Biological Control Systems*. Manchester University Press Manchester.

Whiting, R. C., S. Sackitey, S. Calderone, K. Morely, and J. G. Phillips. 1996. “Model for the Survival of Staphylococcus Aureus in Nongrowth Environments.” *International Journal of Food Microbiology* 31 (1–3): 231–43. https://doi.org/10.1016/0168-1605(96)01002-1.

Widhiati, Suci, Dewajani Purnomosari, Tri Wibawa, and Hardyanto Soebono. 2022. "The Role of Gut Microbiome in Inflammatory Skin Disorders: A Systematic Review." *Dermatology Reports* 14 (1): 9188. https://doi.org/10.4081/dr.2022.9188.

Wiig, Helge, and Melody A. Swartz. 2012. "Interstitial Fluid and Lymph Formation and Transport: Physiological Regulation and Roles in Inflammation and Cancer." *Physiological Reviews* 92 (3): 1005–60. https://doi.org/10.1152/physrev.00037.2011.

Wilde, Aimee D., Daniel J. Snyder, Nicole E. Putnam, Michael D. Valentino, Neal D. Hammer, Zachery R. Lonergan, Scott A. Hinger, et al. 2015. "Bacterial Hypoxic Responses Revealed as Critical Determinants of the Host-Pathogen Outcome by TnSeq Analysis of Staphylococcus Aureus Invasive Infection." *PLOS Pathogens* 11 (12): e1005341. https://doi.org/10.1371/journal.ppat.1005341.

Williams, H., C. Robertson, A. Stewart, N. Aït-Khaled, G. Anabwani, R. Anderson, I. Asher, et al. 1999. "Worldwide Variations in the Prevalence of Symptoms of Atopic Eczema in the International Study of Asthma and Allergies in Childhood." *The Journal of Allergy and Clinical Immunology* 103 (1 Pt 1): 125–38. https://doi.org/10.1016/s0091-6749(99)70536-1.

Williams, Michael R., Stephen K. Costa, Livia S. Zaramela, Shadi Khalil, Daniel A. Todd, Heather L. Winter, James A. Sanford, et al. 2019. "Quorum Sensing between Bacterial Species on the Skin Protects against Epidermal Injury in Atopic Dermatitis." *Science Translational Medicine* 11 (490): eaat8329. https://doi.org/10.1126/scitranslmed.aat8329.

Wohlrab, Johannes, Alexandra Gebert, and Reinhard H. H. Neubert. 2018. "Lipids in the Skin and pH." *pH of the Skin: Issues and Challenges* 54: 64–70. https://doi.org/10.1159/000489519.

Wong, Richard, Stefan Geyer, Wolfgang Weninger, Jean-Claude Guimberteau, and Jason K. Wong. 2016. "The Dynamic Anatomy and Patterning of Skin." *Experimental Dermatology* 25 (2): 92–98. https://doi.org/10.1111/exd.12832.

Wong, Tit-Yee. 2017. "Smog Induces Oxidative Stress and Microbiota Disruption." *Journal of Food and Drug Analysis* 25 (2): 235–44. https://doi.org/10.1016/j.jfda.2017.02.003.

Wong, Waihay J., Theresa Richardson, John T. Seykora, George Cotsarelis, and M Celeste Simon. 2015. "Hypoxia-Inducible Factors Regulate Filaggrin Expression and Epidermal Barrier Function." *Journal of Investigative Dermatology* 135 (2): 454–61. https://doi.org/10.1038/jid.2014.283.

Wu, Yuqing, Yongjie Liu, Erich Gulbins, and Heike Grassmé. 2021. "The Anti-Infectious Role of Sphingosine in Microbial Diseases." *Cells* 10 (5): 1105. https://doi.org/10.3390/cells10051105.

Yanez, Diana A., Richard K. Lacher, Aurobind Vidyarthi, and Oscar R. Colegio. 2017. "The Role of Macrophages in Skin Homeostasis." *Pflugers Archiv : European Journal of Physiology* 469 (3–4): 455–63. https://doi.org/10.1007/s00424-017-1953-7.

Yang, John-Jackson, Ting-Wei Chang, Yong Jiang, Hsin-Jou Kao, Bin-Hao Chiou, Ming-Shan Kao, and Chun-Ming Huang. 2018. "Commensal Staphylococcus Aureus Provokes Immunity to Protect against Skin Infection of Methicillin-Resistant Staphylococcus Aureus." *International Journal of Molecular Sciences* 19 (5): 1290. https://doi.org/10.3390/ijms19051290.

Yang, Lan, Jinrong Fu, and Yufeng Zhou. 2020. "Research Progress in Atopic March." *Frontiers in Immunology* 11: 1907. https://doi.org/10.3389/fimmu.2020.01907.

Yoel, Uri, Jacob Gopas, Janet Ozer, Roni Peleg, and Pesach Shvartzman. 2015. "Canine Scent Detection of Volatile Elements, Characteristic of Malignant Cells, in Cell Cultures." *The Israel Medical Association Journal: IMAJ* 17 (9): 567–70.

Yoshinari, Tomoya, Shohei Sakuda, Maiko Watanabe, Yoichi Kamata, Takahiro Ohnishi, and Yoshiko Sugita-Konishi. 2013. "New Metabolic Pathway for Converting Blasticidin S in Aspergillus Flavus and Inhibitory Activity of Aflatoxin Production by Blasticidin S Metabolites." *Journal of Agricultural and Food Chemistry* 61 (33): 7925–31. https://doi.org/10.1021/jf402745c.

Zainal, Hazleen, Adawiyah Jamil, Norazirah Md Nor, and Min Moon Tang. 2020. "Skin pH Mapping and Its Relationship with Transepidermal Water Loss, Hydration and Disease Severity in Adult Patients with Atopic Dermatitis." *Skin Research and Technology: Official Journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI)* 26 (1): 91–98. https://doi.org/10.1111/srt.12768.

Zeeuwen, Patrick L. J. M., Thomas H. A. Ederveen, Danique A. van der Krieken, Hanna Niehues, Jos Boekhorst, Sanja Kezic, Daniëlle A. T. Hanssen, et al. 2017. "Gram-Positive Anaerobe Cocci Are Underrepresented in the Microbiome of Filaggrin-Deficient Human Skin." *The Journal of Allergy and Clinical Immunology* 139 (4): 1368–71. https://doi.org/10.1016/j.jaci.2016.09.017.

Zeeuwen, Patrick LJM, Jos Boekhorst, Ellen H. van den Bogaard, Heleen D. de Koning, Peter MC van de Kerkhof, Delphine M. Saulnier, Iris I. van Swam, et al. 2012. "Microbiome Dynamics of Human Epidermis Following Skin Barrier Disruption." *Genome Biology* 13 (11): R101. https://doi.org/10.1186/gb-2012-13-11-r101.

Zeng, Jinrong, Jianhua Dou, Lihua Gao, Yaping Xiang, Jinhua Huang, Shu Ding, Jing Chen, et al. 2020. "Topical Ozone Therapy Restores Microbiome Diversity in Atopic Dermatitis." *International Immunopharmacology* 80 (March): 106191. https://doi.org/10.1016/j.intimp.2020.106191.

Zhai, Hongbo, Heidi P. Chan, Sara Farahmand, and Howard I. Maibach. 2009. "Measuring Human Skin Buffering Capacity: An in Vitro Model." *Skin Research and Technology: Official Journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI)* 15 (4): 470–75. https://doi.org/10.1111/j.1600-0846.2009.00392.x.

Zhang, Dan, Liang Zou, Ding-Tao Wu, Qi-Guo Zhuang, Hua-Bin Li, Vuyo Mavumengwana, Harold Corke, and Ren-You Gan. 2021. "Discovery of 1'-Acetoxychavicol Acetate (ACA) as a Promising Antibacterial Compound from Galangal (Alpinia Galanga (Linn.) Willd)." *Industrial Crops and Products* 171: 113883. https://doi.org/10.1016/j.indcrop.2021.113883.

Zhao, Na, Danhong Cheng, Ying Jian, Yao Liu, Junlan Liu, Qian Huang, Lei He, et al. 2021. "Molecular Characteristics of Staphylococcus Aureus Isolates Colonizing Human Nares and Skin." *Medicine in Microecology* 7 (March): 100031. https://doi.org/10.1016/j.medmic.2020.100031.

Zhou, Chunyi, and Paul D. Fey. 2020. "The Acid Response Network of Staphylococcus Aureus." *Current Opinion in Microbiology* 55 (June): 67–73. https://doi.org/10.1016/j.mib.2020.03.006.

Zhu, Yaqian, Nandita Mitra, Yuanqing Feng, Sarah Tishkoff, Ole Hoffstad, and David Margolis. 2021. "Filaggrin Variation Differs for European-Americans and African-Americans." *The Journal of Investigative Dermatology* 141 (7): 1855–57. https://doi.org/10.1016/j.jid.2020.12.022.

Zmantar, Tarek, Bochra Kouidhi, Hanene Miladi, Kacem Mahdouani, and Amina Bakhrouf. 2010. "A Microtiter Plate Assay for Staphylococcus Aureus Biofilm Quantification at Various pH Levels and Hydrogen Peroxide Supplementation." *The New Microbiologica* 33 (2): 137–45.

Zuberbier, T., W. Aberer, R. Asero, A. H. Abdul Latiff, D. Baker, B. Ballmer-Weber, J. A. Bernstein, et al. 2018. "The EAACI/GA^{2}LEN/EDF/WAO Guideline for the Definition, Classification, Diagnosis and Management of Urticaria." *Allergy* 73 (7): 1393–1414. https://doi.org/10.1111/all.13397.

Chapter 7 - Appendix

7.1 Supplementary Figures

7.1.1 Section 2.5 Supplement

Supplement Table 7.1 Oxygen Tolerance List

Genus and species were collected from the AnnotIEM-annotated taxonomy tables from the CK-AD and ProRaD studies. Details regarding how the list was created can be found in section 2.5. Abbreviations: species (SPP).

Genus	Species	Oxygen Tolerance
[Agitococcus] lubricus group	*SPP*	Other
[Aquaspirillum] arcticum group	*SPP*	Other
[Eubacterium] coprostanoligenes group	*SPP*	Other
[Eubacterium] coprostanoligenes group		Other
[Eubacterium] eligens group	*SPP*	Other
[Eubacterium] hallii group	*SPP*	Other
[Eubacterium] nodatum group	*Eubacterium infirmum F0142*	Other
[Eubacterium] nodatum group	*SPP*	Other
[Eubacterium] oxidoreducens group	*SPP*	Other
[Eubacterium] ventriosum group	*human gut SPP*	Other
[Eubacterium] xylanophilum group	*SPP*	Other
[Polyangium] brachysporum group	*[Polyangium] brachysporum*	Other
[Ruminococcus] gauvreauii group	*SPP*	Other
[Ruminococcus] torques group	*SPP*	Other
[Ruminococcus] torques group	*Dorea longicatena*	Other
1174-901-12	*SPP*	Other
1174-901-12	*SPP soil bacterium*	Other
1174-901-12	*SPP Rhizobiales bacterium*	Other
966-1	*SPP*	Other
Abies	*homolepis*	Other
Abiotrophia	*SPP*	Facultative Anaerobic
Abiotrophia	*defectiva*	Facultative Anaerobic
Acetoanaerobium	*SPP*	Anaerobic
Acetobacter	*SPP*	Aerobic
Acetobacter	*pasteurianus*	Other
Acetobacteroides	*SPP*	Anaerobic
Achromobacter	*SPP*	Aerobic
Achromobacter	*xylosoxidans*	Aerobic
Acidibacter	*SPP*	Aerobic
Acidiphilium	*SPP*	Aerobic

Acidipropionibacterium	*acidipropionici*	Facultative Anaerobic
Acidipropionibacterium	*jensenii*	Facultative Anaerobic
Acidisoma	*SPP*	Aerobic
Acidisphaera	*SPP*	Aerobic
Acidovorax	*SPP*	Aerobic
Acidovorax	*wohlfahrtii*	Aerobic
Acidovorax	*defluvii*	Aerobic
Acidovorax	*delafieldii*	Aerobic
Acidovorax	*temperans*	Aerobic
Acidovorax	*soli*	Other
Acidovorax	*caeni*	Facultative Anaerobic
Acidovorax	*oryzae*	Other
Acinetobacter	*lwoffii*	Aerobic
Acinetobacter	*oryzae*	Aerobic
Acinetobacter	*ursingii*	Aerobic
Acinetobacter	*SPP*	Aerobic
Acinetobacter	*pittii*	Aerobic
Acinetobacter	*calcoaceticus*	Aerobic
Acinetobacter	*parvus*	Aerobic
Acinetobacter	*baumannii*	Aerobic
Acinetobacter	*junii*	Aerobic
Acinetobacter	*radioresistens*	Aerobic
Acinetobacter	*guillouiae*	Aerobic
Acinetobacter	*schindleri*	Aerobic
Acinetobacter	*soli*	Aerobic
Acinetobacter	*towneri*	Aerobic
Acinetobacter	*vivianii*	Aerobic
Acinetobacter	*tjernbergiae*	Anaerobic
Acinetobacter	*johnsonii*	Facultative Anaerobic
Acinetobacter	*Clostridium sp. Culture-47*	Other
Actinidia	*chinensis*	Other
Actinobacillus	*SPP*	Aerobic
Actinobacillus	*pleuropneumoniae*	Facultative Anaerobic
Actinobaculum	*massiliense*	Facultative Anaerobic
Actinomyces	*turicensis*	Anaerobic
Actinomyces	*dentalis*	Anaerobic
Actinomyces	*gerencseriae*	Anaerobic
Actinomyces	*lingnae*	Anaerobic
Actinomyces	*meyeri*	Anaerobic
Actinomyces	*naeslundii*	Anaerobic
Actinomyces	*odontolyticus*	Anaerobic
Actinomyces	*radicidentis*	Anaerobic
Actinomyces	*SPP*	Facultative Anaerobic
Actinomyces	*neuii*	Facultative Anaerobic
Actinomyces	*ihumii*	Facultative Anaerobic
Actinomyces	*graevenitzii*	Facultative Anaerobic
Actinomyces	*massiliensis*	Facultative Anaerobic
Actinomyces	*johnsonii*	Facultative Anaerobic

Actinomyces	*viscosus*	Facultative Anaerobic
Actinomycetospora	*chiangmaiensis*	Aerobic
Actinomycetospora	*atypica*	Aerobic
Actinotignum	*urinale*	Anaerobic
Actinotignum	*schaalii*	Anaerobic
Adhaeribacter	*SPP*	Aerobic
Adlercreutzia	*Adlercreutzia*	Anaerobic
Adlercreutzia	*equolifaciens*	Anaerobic
Aegypius	*monachus (black vulture)*	Other
Aeribacillus	*SPP*	Aerobic
Aerococcus	*SPP*	Aerobic
Aerococcus	*christensenii*	Aerobic
Aerococcus	*urinae*	Aerobic
Aerococcus	*urinaeequi*	Aerobic
Aerococcus	*sanguinicola*	Facultative Anaerobic
Aerococcus	*viridans*	Facultative Anaerobic
Aeromicrobium	*SPP*	Aerobic
Aeromicrobium	*fastidiosum*	Aerobic
Aeromicrobium	*alkaliterrae*	Aerobic
Aeromicrobium	*panaciterrae*	Aerobic
Aeromonas	*caviae*	Aerobic
Aeromonas	*SPP*	Facultative Anaerobic
Aeromonas	*media*	Facultative Anaerobic
Aeromonas	*veronii*	Facultative Anaerobic
Aeromonas	*hydrophila*	Facultative Anaerobic
Aeromonas	*salmonicida*	Facultative Anaerobic
Aeromonas	*taiwanensis*	Facultative Anaerobic
Agathobacter	*SPP*	Anaerobic
Aggregatibacter	*aphrophilus*	Facultative Anaerobic
Aggregatibacter	*SPP*	Facultative Anaerobic
Aggregatibacter	*segnis*	Facultative Anaerobic
Aggregatibacter	*Azospirillum sp. 51_20*	Other
Agreia	*SPP*	Aerobic
Agreia	*bicolorata*	Aerobic
Agrobacterium	*radiobacter*	Facultative Anaerobic
Agrococcus	*casei*	Aerobic
Agrococcus	*SPP*	Other
Agrococcus	*citreus*	Other
Agromyces	*aureus*	Aerobic
Agromyces	*SPP*	Aerobic
Agromyces	*cerinus*	Aerobic
Agromyces	*rhizospherae*	Aerobic
Akkermansia	*SPP*	Anaerobic
Akkermansia	*muciniphila*	Anaerobic
Alcaligenes	*faecalis*	Facultative Anaerobic
Alcanivorax	*hongdengensis*	Aerobic
Alcanivorax	*venustensis*	Aerobic
Alicycliphilus	*denitrificans*	Facultative Anaerobic

Aliicoccus	*SPP*	Aerobic
Alishewanella	*SPP*	Facultative Anaerobic
Alishewanella	*agri*	Facultative Anaerobic
Alistipes	*SPP*	Anaerobic
Alistipes	*obesi*	Anaerobic
Alistipes	*onderdonkii*	Anaerobic
Alistipes	*shahii*	Anaerobic
Alistipes	*putredinis*	Anaerobic
Aliterella	*SPP*	Facultative Anaerobic
Alkalibacterium	*gilvum*	Anaerobic
Alkalibacterium	*subtropicum*	Facultative Anaerobic
Alkanindiges	*SPP*	Aerobic
Alkanindiges	*illinoisensis*	Aerobic
Allium	*cepa (onion)*	Other
Alloiococcus	*SPP*	Aerobic
Alloiococcus	*otitis*	Aerobic
Alloprevotella	*SPP*	Anaerobic
Alloprevotella	*rava*	Anaerobic
Alloprevotella	*tannerae*	Anaerobic
Allorhizobium	*vitis*	Aerobic
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	*SPP*	Aerobic
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	*Agrobacterium radiobacter*	Other
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	*Bacillus thuringiensis*	Other
Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	*Agrobacterium sp. Bmb16*	Other
Alloscardovia	*SPP*	Anaerobic
Altererythrobacter	*SPP*	Anaerobic
Altererythrobacter	*sediminis*	Anaerobic
Altererythrobacter	*aestuarii*	Anaerobic
Altererythrobacter	*Starria zimbabweensis SAG 74.90*	Other
Alysiella	*Kingella sp. oral clone ID059*	Other
Amaricoccus	*SPP*	Aerobic
Amaricoccus	*tamworthensis*	Aerobic
Aminobacter	*SPP*	Aerobic
Ammoniphilus	*SPP*	Aerobic
Amnibacterium	*SPP*	Aerobic
Amnibacterium	*kyonggiense*	Aerobic
Amycolatopsis	*SPP*	Aerobic
Anaerobiospirillum	*SPP*	Anaerobic
Anaerobiospirillum	*succiniciproducens*	Anaerobic
Anaerococcus	*octavius*	Anaerobic
Anaerococcus	*pacaensis*	Anaerobic
Anaerococcus	*prevotii*	Anaerobic

Anaerococcus	*hydrogenalis*	Anaerobic
Anaerococcus	*SPP*	Anaerobic
Anaerococcus	*lactolyticus*	Anaerobic
Anaerococcus	*provenciensis*	Anaerobic
Anaerococcus	*vaginalis*	Anaerobic
Anaerococcus	*asaccharolyticus*	Anaerobic
Anaerococcus	*tetradius*	Anaerobic
Anaerococcus	*degeneri*	Anaerobic
Anaerococcus	*murdochii*	Anaerobic
Anaerococcus	*provencensis*	Anaerobic
Anaerococcus	*senegalensis*	Anaerobic
Anaerococcus	*obesiensis*	Other
Anaeroglobus	*SPP*	Anaerobic
Anaerolinea	*SPP*	Anaerobic
Anaeromyxobacter	*SPP*	Facultative Anaerobic
Anaeromyxobacter	*dehalogenans*	Facultative Anaerobic
Anaerosporobacter	*SPP*	Anaerobic
Anaerostipes	*SPP*	Anaerobic
Anaerostipes	*hadrus*	Anaerobic
Anaerotruncus	*SPP*	Anaerobic
Anaerovorax	*SPP*	Anaerobic
Ancylobacter	*SPP*	Aerobic
Aneura mirabilis	*Aneura mirabilis*	Other
Angustibacter	*aerolatus*	Aerobic
Anoxybacillus	*SPP*	Aerobic
Anoxybacillus	*flavithermus*	Aerobic
Antricoccus	*SPP*	Aerobic
Aquabacterium	*fontiphilum*	Aerobic
Aquabacterium	*commune*	Aerobic
Aquabacterium	*olei*	Aerobic
Aquabacterium	*parvum*	Aerobic
Aquabacterium	*SPP*	Facultative Anaerobic
Aquamicrobium	*segne*	Aerobic
Aquamicrobium	*SPP*	Aerobic
Aquicella	*SPP*	Aerobic
Aquihabitans	*daechungensis*	Aerobic
Aquincola	*tertiaricarbonis*	Aerobic
Aquipuribacter	*hungaricus*	Aerobic
Arcanobacterium	*urinimassiliense*	Facultative Anaerobic
Arcanobacterium	*SPP*	Other
Arcobacter	*SPP*	Anaerobic
Arcobacter	*butzleri*	Anaerobic
Arcticibacter	*svalbardensis*	Aerobic
Arcticibacter	*SPP*	Aerobic
Arcticibacter	*SPP Pedobacter sp.*	Other
Arenimonas	*SPP*	Aerobic
Aridibacter	*SPP*	Aerobic
Aridibacter	*famidurans*	Aerobic

Arthrobacter	*oryzae*	Aerobic
Arthrobacter	*SPP*	Aerobic
Arthrobacter	*alpinus*	Aerobic
Arthrobacter	*monumenti*	Aerobic
Arthrobacter	*agilis*	Aerobic
Arthrobacter	*russicus*	Aerobic
Arthrobacter	*sanguinis*	Aerobic
Arthrobacter	*humicola*	Aerobic
Arthrobacter	*pascens*	Aerobic
Arthrobacter	*Rathayibacter festucae*	Other
ASND	*g*	Other
Asparagus	*officinalis (garden asparagus)*	Other
Athetis	*lepigone*	Other
Atopobium	*SPP*	Anaerobic
Atopobium	*minutum*	Anaerobic
Atopobium	*deltae*	Anaerobic
Atopobium	*parvulum*	Anaerobic
Atopobium	*vaginae*	Facultative Anaerobic
Atopococcus	*SPP*	Aerobic
Atopococcus		Other
Atopostipes	*SPP*	Facultative Anaerobic
Aureimonas	*SPP*	Aerobic
Aureimonas	*altamirensis*	Aerobic
Auricoccus	*indicus*	Aerobic
Auritidibacter	*ignavus*	Aerobic
Azoarcus	*SPP*	Anaerobic
Azospira	*SPP*	Aerobic
Azospirillum	*lipoferum*	Anaerobic
Azospirillum	*SPP*	Anaerobic
Azotobacter	*SPP*	Aerobic
B48	*SPP*	Other
Babela	*massiliensis*	Other
Bacillus	*kochii*	Aerobic
Bacillus	*marisflavi*	Aerobic
Bacillus	*pumilus*	Aerobic
Bacillus	*solani*	Aerobic
Bacillus	*SPP*	Other
Bacillus	*velezensis*	Facultative Anaerobic
Bacillus	*cereus*	Facultative Anaerobic
Bacillus	*licheniformis*	Facultative Anaerobic
Bacillus	*subtilis*	Facultative Anaerobic
Bacillus	*megaterium*	Facultative Anaerobic
Bacillus	*circulans*	Facultative Anaerobic
Bacillus	*nealsonii*	Facultative Anaerobic
Bacillus	*horikoshii*	Facultative Anaerobic
Bacillus	*simplex*	Facultative Anaerobic
Bacillus	*lehensis*	Facultative Anaerobic

Bacillus	*niacini*	Facultative Anaerobic
Bacillus	*patagoniensis*	Facultative Anaerobic
Bacillus	*carboniphilus*	Facultative Anaerobic
Bacillus	*halodurans*	Facultative Anaerobic
Bacillus	*oceanisediminis*	Facultative Anaerobic
Bacillus	*flexus*	Facultative Anaerobic
Bacillus	*thuringiensis*	Facultative Anaerobic
Bacillus	*huizhouensis*	Facultative Anaerobic
Bacillus	*humi*	Facultative Anaerobic
Bacillus	*mobilis*	Facultative Anaerobic
Bacillus	*paranthracis*	Facultative Anaerobic
Bacillus	*wiedmannii*	Facultative Anaerobic
Bacillus	*Mycobacterium abscessus subsp. abscessus*	Other
bacterium enrichment culture clone SRC_DSC20	*bacterium enrichment culture clone SRC_DSC20*	Other
Bacteroides	*SPP*	Anaerobic
Bacteroides	*dorei*	Anaerobic
Bacteroides	*uniformis*	Anaerobic
Bacteroides	*ovatus*	Anaerobic
Bacteroides	*eggerthii*	Anaerobic
Bacteroides	*thetaiotaomicron*	Anaerobic
Bacteroides	*finegoldii*	Anaerobic
Bacteroides	*caccae*	Anaerobic
Bacteroides	*coagulans*	Anaerobic
Bacteroides	*coprocola*	Anaerobic
Bacteroides	*massiliensis*	Anaerobic
Bacteroides	*vulgatus*	Anaerobic
Bacteroides	*cellulosilyticus*	Anaerobic
Bacteroides	*fragilis*	Anaerobic
Barnesiella	*SPP*	Anaerobic
Barrientosiimonas	*humi*	Aerobic
Bartonella	*SPP*	Aerobic
Basfia	*Mannheimia massilioguelmaensis*	Other
Bauldia	*consociata*	Aerobic
Bdellovibrio	*SPP*	Aerobic
Bdellovibrio	*bacteriovorus*	Aerobic
Bergeyella	*SPP*	Aerobic
Bergeyella	*zoohelcum*	Anaerobic
Bibersteinia	*SPP*	Facultative Anaerobic
Bifidobacterium	*longum*	Anaerobic
Bifidobacterium	*SPP*	Anaerobic
Bifidobacterium	*breve*	Anaerobic
Bifidobacterium	*animalis*	Anaerobic
Bifidobacterium	*bifidum*	Anaerobic
Bifidobacterium	*adolescentis*	Anaerobic
Bifidobacterium	*pseudocatenulatum*	Anaerobic

Bifidobacterium	*asteroides*	Anaerobic
Bifidobacterium	*angulatum*	Anaerobic
Bifidobacterium	*kashiwanohense*	Anaerobic
Blastocatella	*SPP*	Aerobic
Blastococcus	*endophyticus*	Aerobic
Blastococcus	*saxobsidens*	Aerobic
Blastococcus	*SPP*	Other
Blastopirellula	*SPP*	Aerobic
Blautia	*SPP*	Anaerobic
Blautia	*Marseille*	Anaerobic
Blautia	*glucerasea*	Anaerobic
Blautia	*obeum*	Anaerobic
Blautia	*wexlerae*	Anaerobic
Blautia	*faecis*	Anaerobic
Blautia	*luti*	Anaerobic
Blautia	*Ruminococcus sp. Marseille-P328*	Other
Blautia	*SPP Ruminococcus sp.*	Other
Bordetella	*SPP*	Facultative Anaerobic
Bosea	*SPP*	Aerobic
Bosea	*vestrisii*	Other
Bouteloua dactyloides	*Bouteloua dactyloides*	Other
Brachybacterium	*squillarum*	Aerobic
Brachybacterium	*fresconis*	Aerobic
Brachybacterium	*massiliense*	Aerobic
Brachybacterium	*muris*	Aerobic
Brachybacterium	*paraconglomeratum*	Other
Brachybacterium	*SPP*	Other
Brachybacterium	*xerosis*	Facultative Anaerobic
Brachybacterium	*arcticum*	Facultative Anaerobic
Brachybacterium	*horti*	Facultative Anaerobic
Brachybacterium	*nesterenkovii*	Facultative Anaerobic
Brachymonas	*SPP*	Aerobic
Bradyrhizobium	*SPP*	Aerobic
Bradyrhizobium	*viridifuturi*	Aerobic
Brasilonema	*SPP*	Other
Brassica	*napus (rape)*	Other
Brassica	*nigra (black mustard)*	Other
Brevibacillus	*agri*	Aerobic
Brevibacillus	*parabrevis*	Aerobic
Brevibacillus	*borstelensis*	Aerobic
Brevibacillus	*SPP*	Other
Brevibacterium	*SPP*	Aerobic
Brevibacterium	*ravenspurgense*	Aerobic
Brevibacterium	*celere*	Aerobic
Brevibacterium	*epidermidis*	Aerobic
Brevibacterium	*antiquum*	Aerobic
Brevibacterium	*casei*	Aerobic

Brevibacterium	*linens*	Aerobic
Brevibacterium	*massiliense*	Aerobic
Brevibacterium	*mcbrellneri*	Aerobic
Brevibacterium	*paucivorans*	Aerobic
Brevibacterium	*pityocampae*	Aerobic
Brevibacterium	*sanguinis*	Aerobic
Brevibacterium	*senegalense*	Aerobic
Brevibacterium	*jeotgali*	Aerobic
Brevibacterium	*yomogidense*	Aerobic
Brevundimonas	*albigilva*	Aerobic
Brevundimonas	*SPP*	Aerobic
Brevundimonas	*intermedia*	Aerobic
Brevundimonas	*diminuta*	Aerobic
Brevundimonas	*aveniformis*	Aerobic
Brevundimonas	*basaltis*	Aerobic
Brevundimonas	*mediterranea*	Aerobic
Brevundimonas	*subvibrioides*	Aerobic
Brevundimonas	*vesicularis*	Aerobic
Brevundimonas	*Tibet-IBa1*	Other
Brevundimonas	*Caulobacter sp. FWC14*	Other
Brochothrix	*thermosphacta*	Facultative Anaerobic
Brochothrix	*campestris*	Facultative Anaerobic
Brooklawnia	*SPP*	Facultative Anaerobic
Brumimicrobium	*SPP*	Aerobic
Bryobacter	*SPP*	Aerobic
Bryum	*argenteum var. argenteum*	Other
Buchnera	*aphidicola*	Facultative Anaerobic
Buchnera	*SPP*	Facultative Anaerobic
Bulleidia	*SPP*	Anaerobic
Burkholderia	*SPP*	Aerobic
Burkholderia	*cepacia*	Aerobic
Burkholderia	*puraquae*	Aerobic
Burkholderia-Caballeronia-Paraburkholderia	*gladioli*	Aerobic
Buttiauxella	*gaviniae*	Facultative Anaerobic
Butyricicoccus	*SPP*	Anaerobic
Butyricimonas	*SPP*	Anaerobic
Butyrivibrio	*SPP*	Anaerobic
C39	*SPP*	Other
CAG-352	*SPP*	Other
CAG-56	*SPP*	Other
Caldimonas	*meghalayensis*	Aerobic
Calothrix	*SPP*	Facultative Anaerobic
Camelimonas	*fluminis*	Aerobic
Camelimonas	*SPP*	Aerobic
Camelimonas	*SPP Microvirga sp.*	Other
Campylobacter	*ureolyticus*	Anaerobic
Campylobacter	*SPP*	Anaerobic

Campylobacter	*hominis*	Anaerobic
Campylobacter	*gracilis*	Anaerobic
Campylobacter	*showae*	Anaerobic
Campylobacter	*helveticus*	Anaerobic
Campylobacter	*concisus*	Anaerobic
candidate division SR1 bacterium MGEHA	*candidate division SR1 bacterium MGEHA*	Other
Candidatus	*Saccharibacteria*	Facultative Anaerobic
Candidatus	*Peptoniphilus*	Other
Candidatus	*SPP*	Other
Candidatus Alysiosphaera	*SPP*	Other
Candidatus Cardinium	*Bacteroidetes endosymbiont of Metaseiulus occidentalis*	Other
Candidatus Endomicrobium	*SPP*	Other
Candidatus Finniella	*SPP*	Other
Candidatus Lumbricincola	*SPP*	Other
Candidatus Omnitrophus	*Candidatus Omnitrophus fodinae SCGC AAA011-A17*	Other
Candidatus Paracaedibacter	*endosymbiont of Acanthamoeba sp. KA/E9*	Other
Candidatus Regiella	*Regiella*	Other
Candidatus Saccharimonas	*SPP*	Facultative Anaerobic
Candidatus Uzinura	*Flavobacteriales endosymbiont of Carulaspis juniperi D241*	Other
Candidatus Xiphinematobacter	*SPP*	Other
Cannabis sativa (hemp)	*Cannabis sativa (hemp)*	Other
Capnocytophaga	*SPP*	Facultative Anaerobic
Capnocytophaga	*canimorsus*	Facultative Anaerobic
Capnocytophaga	*haemolytica*	Facultative Anaerobic
Capnocytophaga	*gingivalis*	Facultative Anaerobic
Capnocytophaga	*granulosa*	Facultative Anaerobic
Capnocytophaga	*leadbetteri*	Facultative Anaerobic
Capnocytophaga	*ochracea*	Facultative Anaerobic
Capnocytophaga	*sputigena*	Facultative Anaerobic
Capsicum annuum	*Capsicum annuum*	Other
Capsicum annuum var. glabriusculum	*Capsicum annuum var. glabriusculum*	Other
Cardiobacterium	*SPP*	Aerobic
Carex	*siderosticta*	Other
Carnobacterium	*SPP*	Facultative Anaerobic
Carnobacterium	*divergens*	Facultative Anaerobic
Carnobacterium	*pleistocenium*	Facultative Anaerobic
Carya cathayensis	*Carya cathayensis*	Other
Castanea	*mollissima (Chinese chestnut)*	Other
Catenibacterium	*SPP*	Anaerobic
Catonella	*SPP*	Anaerobic

Catonella	*morbi*	Anaerobic
Caulobacter	*SPP*	Aerobic
Caulobacter	*henricii*	Aerobic
Caulobacter	*segnis*	Aerobic
CCMM	*g*	Other
Cellulomonas	*SPP*	Facultative Anaerobic
Cellulomonas	*pakistanensis*	Facultative Anaerobic
Cellulomonas	*xylanilytica*	Facultative Anaerobic
Cellulosimicrobium	*cellulans*	Facultative Anaerobic
Cellvibrio	*SPP*	Aerobic
Cellvibrio	*vulgaris*	Aerobic
CENA359	*SPP*	Other
Cenchrus americanus	*Cenchrus americanus*	Other
Cercis gigantea	*Cercis gigantea*	Other
Chalicogloea	*SPP*	Facultative Anaerobic
Chamaesiphon	*polonicus*	Facultative Anaerobic
Chiayiivirga	*SPP*	Aerobic
Chlorobaculum	*tepidum*	Anaerobic
Chlorobi	*SPP*	Anaerobic
Chlorobi bacterium canine oral taxon 046	*SPP*	Anaerobic
Christensenella	*SPP*	Anaerobic
Christensenella	*minuta*	Anaerobic
Christensenellaceae	*SPP*	Anaerobic
Christensenellaceae R-7 group	*SPP*	Anaerobic
Chromochloris	*zofingiensis*	Other
Chromohalobacter	*SPP*	Facultative Anaerobic
Chroococcidiopsis	*SPP*	Other
Chryseobacterium	*haifense*	Aerobic
Chryseobacterium	*indologenes*	Aerobic
Chryseobacterium	*SPP*	Aerobic
Chryseobacterium	*luteum*	Aerobic
Chryseobacterium	*hispanicum*	Aerobic
Chryseobacterium	*hominis*	Aerobic
Chryseobacterium	*halperniae*	Aerobic
Chryseobacterium	*scophthalmum*	Aerobic
Chryseobacterium	*bovis*	Aerobic
Chryseobacterium	*letacus*	Aerobic
Chryseobacterium	*koreense*	Aerobic
Chryseobacterium	*indoltheticum*	Aerobic
Chryseobacterium	*isbiliense*	Aerobic
Chryseobacterium	*piscicola*	Aerobic
Chryseobacterium	*rhizoplanae*	Aerobic
Chryseobacterium	*shandongense*	Aerobic
Chryseobacterium	*oleae*	Aerobic
Chryseobacterium	*koreense*	Aerobic
Chryseobacterium	*hispalense*	Aerobic
Chryseobacterium	*massiliae*	Aerobic

Chryseobacterium	*ureilyticum*	Aerobic
Chryseobacterium	*zeae*	Aerobic
Chryseobacterium	*Kaistella flava*	Other
Chryseobacterium	*Flavobacterium sp. 'Smarlab BioMol-2300973'*	Other
Chryseolinea	*SPP*	Aerobic
Chryseomicrobium	*SPP*	Aerobic
Chryseomicrobium	*aureum*	Aerobic
Chryseomicrobium	*Bacillus sp. ChroAq 29*	Other
Chthoniobacter	*SPP*	Aerobic
Citricoccus	*Micrococcus luteus*	Other
Citricoccus	*Micrococcus sp. m2-21*	Other
Citrobacter	*pasteurii*	Anaerobic
Citrobacter	*freundii*	Facultative Anaerobic
Citrobacter	*SPP*	Facultative Anaerobic
Citrobacter	*portucalensis*	Facultative Anaerobic
Citrullus lanatus subsp. vulgaris (watermelon)	*Citrullus lanatus subsp. vulgaris (watermelon)*	Other
CL500-29 marine group	*SPP*	Other
Clavibacter	*michiganensis subsp. michiganensis*	Aerobic
Clavibacter	*michiganensis*	Aerobic
Cloacibacterium	*rupense*	Aerobic
Cloacibacterium	*SPP*	Facultative Anaerobic
Cloacibacterium	*normanense*	Facultative Anaerobic
Clostridiisalibacter	*Senegalia massiliensis*	Other
Clostridioides	*difficile*	Anaerobic
Clostridium	*sensu stricto*	Anaerobic
Clostridium	*sensu stricto*	Anaerobic
Clostridium	*SPP*	Anaerobic
Clostridium	*bornimense*	Anaerobic
Clostridium	*citroniae*	Anaerobic
Clostridium	*disporicum*	Anaerobic
Clostridium	*innocuum*	Anaerobic
Clostridium	*nitrophenolicum*	Anaerobic
Clostridium	*oceanicum*	Anaerobic
Clostridium	*ramosum*	Anaerobic
Clostridium	*saccharobutylicum*	Anaerobic
Clostridium	*saccharoperbutylacetonicum*	Anaerobic
Clostridium	*ventriculi*	Anaerobic
Clostridium	*clostridioforme*	Anaerobic
Clostridium	*saudiense*	Anaerobic
Clostridium	*sensu stricto*	Anaerobic
Clostridium sensu stricto	*SPP*	Anaerobic
Cnuella	*SPP*	Aerobic
Cobetia	*pacifica*	Aerobic
Coccomyxa sp. LA000219	*Coccomyxa sp. LA000219*	Other
Cocos	*nucifera (coconut palm)*	Other

Cohnella	*SPP*	Facultative Anaerobic
Collinsella	*aerofaciens*	Anaerobic
Collinsella	*SPP*	Facultative Anaerobic
Colwellia	*SPP*	Facultative Anaerobic
Comamonas	*denitrificans*	Aerobic
Comamonas	*SPP*	Aerobic
Comamonas	*terrigena*	Aerobic
Comamonas	*aquatica*	Aerobic
Comamonas	*testosteroni*	Aerobic
Comamonas	*phosphati*	Facultative Anaerobic
Conchiformibius	*SPP*	Aerobic
Conexibacter	*SPP*	Aerobic
Conyzicola	*lurida*	Aerobic
Coprobacillus	*SPP*	Anaerobic
Coprobacillus	*cateniformis*	Anaerobic
Coprococcus	*eutactus*	Anaerobic
Coprococcus	*SPP*	Anaerobic
Coprococcus	*comes*	Anaerobic
Coprococcus	*catus*	Anaerobic
Coprococcus	*comes*	Anaerobic
Coprothermobacter	*SPP*	Anaerobic
Corchorus capsularis	*capsularis*	Other
Coriobacteriales	*SPP*	Other
Corticibacter	*SPP*	Aerobic
Corticibacterium	*SPP*	Aerobic
Corynebacterium	*tuscaniense*	Aerobic
Corynebacterium	*halotolerans*	Aerobic
Corynebacterium	*variabile*	Aerobic
Corynebacterium	*maris*	Aerobic
Corynebacterium	*glyciniphilum*	Aerobic
Corynebacterium	*bovis*	Aerobic
Corynebacterium	*glucuronolyticum*	Aerobic
Corynebacterium	*lactis*	Aerobic
Corynebacterium	*lipophiloflavum*	Aerobic
Corynebacterium	*urealyticum*	Aerobic
Corynebacterium	*auriscanis*	Aerobic
Corynebacterium	*gottingense*	Aerobic
Corynebacterium	*phoceense*	Aerobic
Corynebacterium	*tuberculostearicum*	Facultative Anaerobic
Corynebacterium	*aurimucosum*	Facultative Anaerobic
Corynebacterium	*simulans*	Facultative Anaerobic
Corynebacterium	*imitans*	Facultative Anaerobic
Corynebacterium	*xerosis*	Facultative Anaerobic
Corynebacterium	*afermentans*	Facultative Anaerobic
Corynebacterium	*amycolatum*	Facultative Anaerobic
Corynebacterium	*appendicis*	Facultative Anaerobic
Corynebacterium	*aquatimens*	Facultative Anaerobic
Corynebacterium	*jeikeium*	Facultative Anaerobic

Corynebacterium	*coyleae*	Facultative Anaerobic
Corynebacterium	*mucifaciens*	Facultative Anaerobic
Corynebacterium	*pilbarense*	Facultative Anaerobic
Corynebacterium	*accolens*	Facultative Anaerobic
Corynebacterium	*suicordis*	Facultative Anaerobic
Corynebacterium	*ihumii*	Facultative Anaerobic
Corynebacterium	*genitalium*	Facultative Anaerobic
Corynebacterium	*pyruviciproducens*	Facultative Anaerobic
Corynebacterium	*kroppenstedtii*	Facultative Anaerobic
Corynebacterium	*pseudogenitalium*	Facultative Anaerobic
Corynebacterium	*casei*	Facultative Anaerobic
Corynebacterium	*pseudodiphtheriticum*	Facultative Anaerobic
Corynebacterium	*bouchesdurhonense*	Facultative Anaerobic
Corynebacterium	*glaucum*	Facultative Anaerobic
Corynebacterium	*vitaeruminis*	Facultative Anaerobic
Corynebacterium	*massiliense*	Facultative Anaerobic
Corynebacterium	*faecale*	Facultative Anaerobic
Corynebacterium	*frankenforstense*	Facultative Anaerobic
Corynebacterium	*atypicum*	Facultative Anaerobic
Corynebacterium	*diphtheriae*	Facultative Anaerobic
Corynebacterium	*durum*	Facultative Anaerobic
Corynebacterium	*glutamicum*	Facultative Anaerobic
Corynebacterium	*mustelae*	Facultative Anaerobic
Corynebacterium	*callunae*	Facultative Anaerobic
Corynebacterium	*efficiens*	Facultative Anaerobic
Corynebacterium	*falsenii*	Facultative Anaerobic
Corynebacterium	*jeddahense*	Facultative Anaerobic
Corynebacterium	*matruchotii*	Facultative Anaerobic
Corynebacterium	*minutissimum*	Facultative Anaerobic
Corynebacterium	*mycetoides*	Facultative Anaerobic
Corynebacterium	*riegelii*	Facultative Anaerobic
Corynebacterium	*singulare*	Facultative Anaerobic
Corynebacterium	*striatum*	Facultative Anaerobic
Corynebacterium	*tapiri*	Facultative Anaerobic
Corynebacterium	*thomssenii*	Facultative Anaerobic
Corynebacterium	*ureicelerivorans*	Facultative Anaerobic
Corynebacterium	*macginleyi*	Facultative Anaerobic
Corynebacterium	*SPP*	Other
Corynebacterium	*HMSC072A02*	Other
Costus pictus	*Costus pictus*	Other
Criibacterium bergeronii	*Criibacterium bergeronii*	Other
Crinalium	*epipsammum*	Facultative Anaerobic
Crossiella	*SPP*	Aerobic
Cucumis	*melo (muskmelon)*	Other
Cucumis	*sativus (cucumber)*	Other
Cucurbita pepo		Other
Cupriavidus	*SPP*	Aerobic
Cupriavidus	*metallidurans*	Facultative Anaerobic

Curtobacterium	*SPP*	Aerobic
Curtobacterium	*flaccumfaciens*	Aerobic
Curtobacterium	*pusillum*	Aerobic
Curvibacter	*delicatus*	Aerobic
Curvibacter	*gracilis*	Aerobic
Curvibacter	*lanceolatus*	Aerobic
Curvibacter	*SPP*	Other
Cutibacterium	*granulosum*	Anaerobic
Cutibacterium	*acnes*	Facultative Anaerobic
Cutibacterium	*SPP*	Facultative Anaerobic
Cutibacterium	*Lepisosteus oculatus (spotted gar)*	Other
Cutibacterium	*Coregonus lavaretus (common whitefish)*	Other
Cypripedium	*macranthos*	Other
Cystobacter	*fuscus*	Aerobic
Cytophaga	*aurantiaca*	Aerobic
Cytophaga	*hutchinsonii*	Aerobic
Cytophaga	*aurantiaca DSM 3654*	Other
Cytophaga	*SPP*	Other
CYUJ	*g*	Other
Dechloromonas	*SPP*	Facultative Anaerobic
Deinococcus	*aquatilis*	Aerobic
Deinococcus	*antarcticus*	Aerobic
Deinococcus	*SPP*	Aerobic
Deinococcus	*radiodurans*	Aerobic
Deinococcus	*geothermalis*	Aerobic
Deinococcus	*radiophilus*	Aerobic
Deinococcus	*metalli*	Aerobic
Deinococcus	*wulumuqiensis*	Aerobic
Delftia	*SPP*	Aerobic
Delftia	*tsuruhatensis*	Other
Dermabacter	*SPP*	Facultative Anaerobic
Dermabacter	*vaginalis*	Facultative Anaerobic
Dermabacter	*hominis*	Facultative Anaerobic
Dermacoccus	*SPP*	Aerobic
Dermacoccus	*nishinomiyaensis*	Aerobic
Desemzia	*incerta*	Aerobic
Desemzia	*SPP*	Anaerobic
Desmochloris	*halophila*	Other
Desulfocapsa	*SPP*	Anaerobic
Desulfotomaculum	*SPP*	Anaerobic
Desulfovibrio	*SPP*	Anaerobic
Devosia	*SPP*	Aerobic
Devosia	*insulae*	Aerobic
Devosia	*riboflavina*	Aerobic
dgA-11 gut group	*SPP*	Other
Dialister	*SPP*	Anaerobic

Dialister	*pneumosintes*	Anaerobic
Dialister	*invisus*	Anaerobic
Dialister	*micraerophilus*	Anaerobic
Dialister	*propionicifaciens*	Anaerobic
Dialister	*Veillonellaceae bacterium DNF00626*	Other
Diaphorobacter	*nitroreducens*	Facultative Anaerobic
Diaphorobacter	*polyhydroxybutyrativorans*	Facultative Anaerobic
Dielma	*SPP*	Anaerobic
Dietzia	*timorensis*	Aerobic
Dietzia	*SPP*	Aerobic
Dietzia	*maris*	Aerobic
Dietzia	*papillomatosis*	Aerobic
Dietzia	*schimae*	Aerobic
Dinghuibacter	*SPP*	Aerobic
Diplosphaera	*SPP Desulfococcus sp.*	Other
DNF00809	*Coriobacteriales bacterium DNF00809*	Other
Dokdonella	*SPP*	Aerobic
Dolosigranulum	*SPP*	Facultative Anaerobic
Dolosigranulum	*pigrum*	Facultative Anaerobic
Domibacillus	*SPP*	Aerobic
Dongia	*SPP*	Aerobic
Dorea	*SPP*	Anaerobic
Dorea	*formicigenerans*	Anaerobic
Dorea	*longicatena*	Anaerobic
DSSD61	*SPP*	Other
Duganella	*SPP*	Aerobic
Duganella	*zoogloeoides*	Aerobic
Dyadobacter	*SPP*	Aerobic
Dyadobacter	*psychrophilus*	Aerobic
Dysgonomonas	*SPP*	Facultative Anaerobic
Dysgonomonas	*capnocytophagoides*	Facultative Anaerobic
Eggerthella	*lenta*	Anaerobic
Eggerthia	*catenaformis*	Anaerobic
Eikenella	*SPP*	Facultative Anaerobic
Eisenbergiella	*SPP*	Anaerobic
Ellin6055	*SPP*	Other
Elusimicrobium	*SPP*	Anaerobic
Empedobacter	*falsenii*	Aerobic
Empedobacter	*brevis*	Aerobic
Empedobacter	*SPP*	Aerobic
Endozoicomonas	*SPP*	Facultative Anaerobic
Enhydrobacter	*SPP*	Anaerobic
Enhydrobacter	*aerosaccus*	Facultative Anaerobic
Enhydrobacter	*Moraxella osloensis*	Other
Enterobacter	*hormaechei*	Aerobic
Enterobacter	*SPP*	Facultative Anaerobic

Enterobacter	*ludwigii*	Facultative Anaerobic
Enterobacter	*cloacae*	Facultative Anaerobic
Enterococcus	*cecorum*	Facultative Anaerobic
Enterococcus	*faecalis*	Facultative Anaerobic
Enterococcus	*gallinarum*	Facultative Anaerobic
Enterococcus	*faecium*	Facultative Anaerobic
Enterococcus	*SPP*	Facultative Anaerobic
Enterococcus	*mundtii*	Facultative Anaerobic
Enterococcus	*casseliflavus*	Facultative Anaerobic
Enterococcus	*pallens*	Facultative Anaerobic
Enterococcus	*raffinosus*	Facultative Anaerobic
Enterorhabdus	*SPP*	Anaerobic
Eremococcus	*SPP*	Facultative Anaerobic
Eremococcus	*coleocola*	Facultative Anaerobic
Eremococcus	*SPP Facklamia sp.*	Other
Erwinia	*SPP*	Facultative Anaerobic
Erwinia	*persicina*	Facultative Anaerobic
Erwinia	*dispersa*	Facultative Anaerobic
Erwinia	*uzenensis*	Facultative Anaerobic
Erwinia	*billingiae*	Other
Erysipelatoclostridium	*SPP*	Anaerobic
Erysipelatoclostridium	*Massiliomicrobiota timonensis*	Other
Erysipelatoclostridium	*Clostridiales bacterium VE202-01*	Other
Erysipelothrix	*SPP*	Other
Erysipelotrichaceae	*SPP*	Facultative Anaerobic
Erythrobacter	*SPP*	Aerobic
Escherichia	*coli*	Facultative Anaerobic
Escherichia-Shigella	*coli*	Facultative Anaerobic
Escherichia-Shigella	*oxytoca*	Facultative Anaerobic
Escherichia-Shigella	*SPP*	Facultative Anaerobic
Ethanoligenens	*SPP*	Anaerobic
Eubacterium	*eligens*	Anaerobic
Eubacterium	*hallii*	Anaerobic
Eubacterium	*ramulus*	Anaerobic
Eubacterium	*siraeum*	Anaerobic
Eubacterium	*SPP*	Anaerobic
Eubacterium	*tenue*	Anaerobic
Eubacterium	*infirmum*	Anaerobic
Eupomatia laurina	*Eupomatia laurina*	Other
Euzebyella	*SPP*	Aerobic
Exiguobacterium	*SPP*	Facultative Anaerobic
Exiguobacterium	*profundum*	Facultative Anaerobic
Exiguobacterium	*aurantiacum*	Facultative Anaerobic
Exiguobacterium	*indicum*	Facultative Anaerobic
Exiguobacterium	*undae*	Facultative Anaerobic
Ezakiella	*coagulans*	Anaerobic

Ezakiella	*peruensis*	Anaerobic
Ezakiella	*SPP*	Anaerobic
Ezakiella	*massiliensis*	Anaerobic
Ezakiella	*Fenollaria timonensis*	Other
Ezakiella	*Tissierellia bacterium S7-1-4*	Other
F0058	*SPP*	Other
F0332	*SPP Actinomyces sp.*	Other
F0332	*SPP*	Other
Facklamia	*SPP*	Facultative Anaerobic
Facklamia	*hominis*	Facultative Anaerobic
Facklamia	*ignava*	Facultative Anaerobic
Facklamia	*languida*	Facultative Anaerobic
Facklamia	*tabacinasalis*	Facultative Anaerobic
Faecalibacterium	*prausnitzii*	Anaerobic
Faecalibacterium	*SPP*	Anaerobic
Faecalicatena	*orotica*	Anaerobic
Falsirhodobacter	*deserti*	Aerobic
Falsirhodobacter	*halotolerans*	Aerobic
Family XIII AD3011 group	*SPP*	Other
Fastidiosipila	*SPP*	Anaerobic
Fenollaria	*massiliensis*	Anaerobic
Fermentimonas	*SPP*	Facultative Anaerobic
Ferruginibacter	*SPP*	Aerobic
FFCH7168	*SPP*	Other
Fibrella	*SPP*	Facultative Anaerobic
Fibrobacter	*SPP*	Anaerobic
Filifactor	*SPP*	Anaerobic
Filifactor	*villosus*	Anaerobic
Fimbriimonas	*SPP*	Aerobic
Finegoldia	*magna*	Anaerobic
Finegoldia	*SPP*	Anaerobic
Flaviaesturariibacter	*SPP*	Aerobic
Flaviflexus	*SPP*	Facultative Anaerobic
Flavisolibacter	*SPP*	Aerobic
Flavitalea	*SPP*	Aerobic
Flavobacterium	*chungbukense*	Aerobic
Flavobacterium	*rivuli*	Aerobic
Flavobacterium	*aquatile*	Aerobic
Flavobacterium	*qiangtangense*	Aerobic
Flavobacterium	*frigidarium*	Aerobic
Flavobacterium	*cutihirudinis*	Aerobic
Flavobacterium	*chungangense*	Aerobic
Flavobacterium	*urocaniciphilum*	Aerobic
Flavobacterium	*hercynium*	Aerobic
Flavobacterium	*fluvii*	Aerobic
Flavobacterium	*psychrophilum*	Aerobic
Flavobacterium	*seoulense*	Aerobic

Flavobacterium	*suncheonense*	Aerobic
Flavobacterium	*segetis*	Aerobic
Flavobacterium	*hibernum*	Aerobic
Flavobacterium	*luticocti*	Aerobic
Flavobacterium	*pectinovorum*	Aerobic
Flavobacterium	*plurextorum*	Aerobic
Flavobacterium	*SPP*	Other
Flavobacterium	*sasangense*	Facultative Anaerobic
Flavobacterium	*aquicola*	Facultative Anaerobic
Flavobacterium	*lindanitolerans*	Facultative Anaerobic
Flavobacterium	*succinicans*	Facultative Anaerobic
Flavonifractor	*plautii*	Anaerobic
Flectobacillus	*SPP*	Aerobic
Flexibacter	*SPP*	Aerobic
Fluviicola	*SPP*	Aerobic
Fodinicola	*SPP*	Aerobic
Fonticella	*SPP*	Anaerobic
Fonticella	*tunisiensis*	Anaerobic
Formivibrio	*SPP*	Anaerobic
Frederiksenia	*SPP*	Aerobic
Fretibacterium	*SPP*	Anaerobic
Friedmanniella	*SPP*	Aerobic
Friedmanniella	*luteola*	Aerobic
Friedmanniella	*sagamiharensis*	Aerobic
Friedmanniella	*antarctica*	Aerobic
Friedmanniella	*capsulata*	Aerobic
Friedmanniella	*spumicola*	Aerobic
Frigoribacterium	*SPP*	Anaerobic
Frisingicoccus	*caecimuris*	Anaerobic
Frondihabitans	*SPP*	Aerobic
FukuN18 freshwater group	*SPP*	Other
Fusibacter	*Peptostreptococcaceae bacterium feline oral taxon 068*	Other
Fusicatenibacter	*saccharivorans*	Anaerobic
Fusicatenibacter	*SPP*	Anaerobic
Fusobacterium	*SPP*	Anaerobic
Fusobacterium	*massiliense*	Anaerobic
Fusobacterium	*nucleatum*	Anaerobic
Fusobacterium	*periodonticum*	Anaerobic
Fusobacterium	*naviforme*	Anaerobic
Fusobacterium	*necrophorum*	Anaerobic
Gaiella	*SPP*	Aerobic
Gallicola	*SPP*	Anaerobic
Gallicola	*Lagierella massiliensis*	Other
Gardnerella	*vaginalis*	Anaerobic
GCA-900066225	*SPP*	Other
Gelidibacter	*SPP*	Aerobic

Gelidibacter	*algens*	Aerobic
Gemella	*morbillorum*	Anaerobic
Gemella	*SPP*	Facultative Anaerobic
Gemella	*haemolysans*	Facultative Anaerobic
Gemella	*palaticanis*	Facultative Anaerobic
Gemella	*sanguinis*	Facultative Anaerobic
Gemmata	*SPP*	Aerobic
Gemmatimonas	*SPP*	Aerobic
Gemmatirosa	*SPP*	Facultative Anaerobic
Genus	*Species*	Oxygen Tolerance
Geobacillus	*SPP*	Other
Geodermatophilus	*brasiliensis*	Aerobic
Georgenia	*SPP*	Facultative Anaerobic
Geranium brycei	*Geranium brycei*	Other
Gillisia	*hiemivivida*	Aerobic
Gillisia	*SPP*	Aerobic
Gillisia	*illustrilutea*	Aerobic
Globicatella	*SPP*	Aerobic
Globicatella	*sanguinis*	Facultative Anaerobic
Gluconobacter	*cerinus*	Aerobic
Gluconobacter	*oxydans*	Aerobic
Glutamicibacter	*SPP*	Aerobic
Glutamicibacter	*arilaitensis*	Aerobic
Glutamicibacter	*nicotianae*	Aerobic
Glutamicibacter	*Arthrobacter sp. M18-2*	Other
Glutamicibacter	*SPP Arthrobacter sp.*	Other
Gordonia	*SPP*	Aerobic
Gordonia	*paraffinivorans*	Aerobic
Gordonia	*sputi*	Aerobic
Gordonia	*polyisoprenivorans*	Aerobic
Gordonia	*lacunae*	Aerobic
Gordonia	*terrae*	Aerobic
Gordonia	*desulfuricans*	Aerobic
Gordonibacter	*urolithinfaciens*	Anaerobic
Gracilibacteria bacterium canine oral taxon 323	*Gracilibacteria bacterium canine oral taxon 323*	Other
Gracilibacteria bacterium oral taxon 872	*Gracilibacteria bacterium oral taxon 872*	Other
Granulicatella	*SPP*	Facultative Anaerobic
Granulicatella	*adiacens*	Facultative Anaerobic
Granulicatella	*elegans*	Facultative Anaerobic
Granulicella	*SPP*	Facultative Anaerobic
Gulosibacter	*chungangensis*	Aerobic
gut metagenome	*gut SPP*	Other
Haematobacter	*missouriensis*	Aerobic
Haematobacter	*massiliensis*	Aerobic
Haematomicrobium	*sanguinis*	Aerobic
Haemophilus	*SPP*	Aerobic

Haemophilus	*paraurethrae*	Aerobic
Haemophilus	*aegyptius*	Aerobic
Haemophilus	*sputorum*	Aerobic
Haemophilus	*influenzae*	Anaerobic
Haemophilus	*parahaemolyticus*	Anaerobic
Haemophilus	*parainfluenzae*	Anaerobic
Haemophilus	*paraphrohaemolyticus*	Anaerobic
Haemophilus	*haemolyticus*	Facultative Anaerobic
Haemophilus	*haemoglobinophilus*	Facultative Anaerobic
Haliangium	*SPP*	Aerobic
Haliangium	*Nannocystineae*	Aerobic
Haliscomenobacter	*hydrossis*	Aerobic
Haliscomenobacter	*SPP*	Aerobic
Halocella	*SPP*	Anaerobic
Halomonas	*stevensii*	Aerobic
Halomonas	*SPP*	Facultative Anaerobic
Halomonas	*titanicae*	Facultative Anaerobic
Haloplasma	*SPP*	Anaerobic
Hamiltonella	*defensa*	Other
Helcococcus	*SPP*	Facultative Anaerobic
Helcococcus	*sueciensis*	Facultative Anaerobic
Helicobacter	*canis*	Anaerobic
Herbaspirillum	*autotrophicum*	Anaerobic
Herbaspirillum	*rubrisubalbicans*	Anaerobic
Herbiconiux	*SPP*	Aerobic
Herbiconiux	*flava*	Aerobic
Herbinix	*luporum*	Anaerobic
Herpetosiphon	*SPP*	Aerobic
Heveochlorella	*hainangensis*	Other
Hirschia	*SPP*	Aerobic
hoa5-07d05 gut group	*SPP*	Other
Holdemanella	*SPP*	Anaerobic
Howardella	*SPP*	Anaerobic
Huanghella	*arctica*	Aerobic
Humibacillus	*Terracoccus sp. S23303*	Other
Humulus lupulus var. lupulus	*Humulus lupulus var. lupulus*	Other
Hungatella	*hathewayi*	Anaerobic
Hyalangium	*minutum*	Other
Hydrobacter	*penzbergensis*	Aerobic
Hydrocarboniphaga	*SPP*	Aerobic
Hydrogenispora	*SPP*	Anaerobic
Hydrogenophaga	*SPP*	Aerobic
Hydrogenophaga	*taeniospiralis*	Aerobic
Hydrogenophilus	*thermoluteolus*	Aerobic
Hydrogenophilus	*SPP*	Facultative Anaerobic
Hymenobacter	*SPP*	Aerobic
Hymenobacter	*glacieicola*	Aerobic

Hymenobacter	*psychrotolerans*	Aerobic
Hymenobacter	*chitinivorans*	Aerobic
Hymenobacter	*metalli*	Aerobic
Hymenobacter	*swuensis*	Aerobic
Hyphomicrobium	*SPP*	Aerobic
Hypnocyclicus	*SPP*	Anaerobic
Iamia	*SPP*	Aerobic
Ideonella	*Cenchrus americanus*	Other
Ignavigranum	*SPP*	Facultative Anaerobic
Ilumatobacter	*SPP*	Aerobic
IMCC26207	*SPP*	Other
Imtechella	*halotolerans*	Aerobic
Incertae Sedis	*SPP*	Anaerobic
Incertae Sedis	*difficile*	Facultative Anaerobic
Intestinibacter	*SPP*	Anaerobic
Intestinibacter	*bartlettii*	Anaerobic
Ipomoea	*batatas (sweet potato)*	Other
IS-44	*SPP*	Other
Izhakiella	*Dickeya chrysanthemi*	Other
Janibacter	*SPP*	Aerobic
Janibacter	*anophelis*	Aerobic
Janibacter	*hoylei*	Aerobic
Janibacter	*terrae*	Aerobic
Janibacter	*indicus*	Aerobic
Janthinobacterium	*SPP*	Aerobic
Janthinobacterium	*agaricidamnosum*	Aerobic
Janthinobacterium	*psychrotolerans*	Facultative Anaerobic
Janthinobacterium	*Dictyostelium polycarpum*	Other
Jatropha curcas	*Jatropha curcas*	Other
Jatrophihabitans	*Frankineae*	Aerobic
Jatrophihabitans	*SPP*	Aerobic
Jeotgalibaca	*SPP*	Aerobic
Jeotgalicoccus	*aerolatus*	Aerobic
Jeotgalicoccus	*SPP*	Aerobic
Jeotgalicoccus	*halotolerans*	Facultative Anaerobic
Jeotgalicoccus	*huakuii*	Facultative Anaerobic
Jeotgalicoccus	*marinus*	Facultative Anaerobic
JGI 0001001-H03	*SPP*	Other
JGI 0001001-H03	*SPP Acidobacteria bacterium*	Other
Johnsonella	*SPP*	Anaerobic
Jonesia	*denitrificans*	Aerobic
Jonquetella	*anthropi*	Anaerobic
JPZU_g		Other
JRNA	*g*	Other
Kaistia	*granuli*	Aerobic
Kallipyga	*massiliensis*	Anaerobic
Kallotenue	*SPP*	Aerobic

Ketogulonicigenium	*vulgare*	Facultative Anaerobic
Kineococcus	*SPP*	Aerobic
Kineococcus	*radiotolerans*	Aerobic
Kineosporia	*rhamnosa*	Aerobic
Kingella	*oralis*	Other
Kingella	*SPP*	Other
Kingella	*denitrificans*	Other
Klebsiella	*oxytoca*	Facultative Anaerobic
Klebsiella	*SPP*	Facultative Anaerobic
Klebsiella	*Enterobacter sp. NISOC_03*	Facultative Anaerobic
Klebsiella	*pneumoniae*	Facultative Anaerobic
Klebsormidium flaccidum	*Klebsormidium flaccidum*	Other
Kluyvera	*intermedia*	Facultative Anaerobic
Kluyvera	*ascorbata*	Facultative Anaerobic
Kluyvera	*georgiana*	Facultative Anaerobic
Knoellia	*SPP*	Aerobic
Knoellia	*flava*	Aerobic
Knoellia	*aerolata*	Aerobic
Knoellia	*subterranea*	Aerobic
Knoellia	*locipacati*	Aerobic
Kocuria	*rhizophila*	Aerobic
Kocuria	*palustris*	Aerobic
Kocuria	*salsicia*	Aerobic
Kocuria	*carniphila*	Aerobic
Kocuria	*rosea*	Aerobic
Kocuria	*gwangalliensis*	Aerobic
Kocuria	*himachalensis*	Aerobic
Kocuria	*koreensis*	Aerobic
Kocuria	*kristinae*	Aerobic
Kocuria	*marina*	Aerobic
Kocuria	*atrinae*	Aerobic
Kocuria	*SPP*	Other
Kosakonia	*cowanii*	Facultative Anaerobic
Kosakonia	*radicincitans*	Facultative Anaerobic
Koukoulia	*SPP*	Aerobic
Kribbella	*SPP*	Aerobic
Kurthia	*SPP*	Facultative Anaerobic
Kurthia	*massiliensis*	Facultative Anaerobic
Kurthia	*huakuii*	Facultative Anaerobic
Kytococcus	*SPP*	Aerobic
Kytococcus	*sedentarius*	Aerobic
Labilithrix	*SPP*	Aerobic
Laceyella	*sacchari*	Aerobic
Lachnoanaerobaculum	*SPP*	Anaerobic
Lachnoanaerobaculum	*umeaense*	Anaerobic
Lachnoanaerobaculum	*orale*	Anaerobic
Lachnoclostridium	*SPP*	Anaerobic
Lachnospira	*SPP*	Anaerobic

Lachnospiraceae	*SPP*	Anaerobic
Lacibacter	*SPP*	Aerobic
Lactobacillus	*crispatus*	Anaerobic
Lactobacillus	*delbrueckii*	Anaerobic
Lactobacillus	*SPP*	Facultative Anaerobic
Lactobacillus	*paracasei*	Facultative Anaerobic
Lactobacillus	*graminis*	Facultative Anaerobic
Lactobacillus	*iners*	Facultative Anaerobic
Lactobacillus	*rhamnosus*	Facultative Anaerobic
Lactobacillus	*sakei*	Facultative Anaerobic
Lactobacillus	*plantarum*	Facultative Anaerobic
Lactobacillus	*psittaci*	Facultative Anaerobic
Lactobacillus	*algidus*	Facultative Anaerobic
Lactobacillus	*jensenii*	Facultative Anaerobic
Lactobacillus	*helveticus*	Facultative Anaerobic
Lactobacillus	*fuchuensis*	Facultative Anaerobic
Lactobacillus	*fermentum*	Facultative Anaerobic
Lactobacillus	*gasseri*	Facultative Anaerobic
Lactobacillus	*acidipiscis*	Facultative Anaerobic
Lactobacillus	*casei*	Facultative Anaerobic
Lactobacillus	*curvatus*	Facultative Anaerobic
Lactobacillus	*kefiri*	Facultative Anaerobic
Lactobacillus	*reuteri*	Facultative Anaerobic
Lactobacillus	*rogosae*	Facultative Anaerobic
Lactococcus	*SPP*	Facultative Anaerobic
Lactococcus	*lactis*	Facultative Anaerobic
Lactococcus	*garvieae*	Facultative Anaerobic
Lactococcus	*laudensis*	Facultative Anaerobic
Lacunisphaera	*SPP*	Other
Lampropedia	*puyangensis*	Aerobic
Lapillicoccus	*jejuensis*	Aerobic
LARJ_g		Other
Larkinella	*SPP*	Aerobic
Lathyrus	*pubescens*	Other
Lautropia	*SPP*	Facultative Anaerobic
Lautropia	*mirabilis*	Facultative Anaerobic
Lawsonella	*clevelandensis*	Anaerobic
Lawsonella	*SPP*	Anaerobic
LB3-76	*SPP Antarctic bacterium LB3-7*	Other
LB3-76	*SPP*	Other
LD29	*SPP*	Other
Legionella	*SPP*	Aerobic
Leifsonia	*SPP*	Aerobic
Leptolyngbya	*SPP*	Facultative Anaerobic
Leptolyngbya	*frigida*	Facultative Anaerobic
Leptolyngbya	*antarctica*	Other
Leptolyngbya	*subtilissima*	Other

Leptolyngbya Es-Yyy1000	*Pseudophormidium sp. WJT25-NPBG1*	Other
Leptolyngbya PCC-6306	*Plectonema sp. SAG 38.90*	Other
Leptothrix	*SPP*	Aerobic
Leptotrichia	*SPP*	Anaerobic
Leptotrichia	*wadei*	Anaerobic
Leptotrichia	*SPP Leptotrichia sp.*	Anaerobic
Leptotrichia	*goodfellowii*	Anaerobic
Leptotrichia	*buccalis*	Anaerobic
Leptotrichia	*hofstadii*	Anaerobic
Leptotrichia	*shahii*	Anaerobic
Leptotrichia	*trevisanii*	Anaerobic
Leptotrichia	*amnionii*	Anaerobic
Leucobacter	*tardus*	Aerobic
Leucobacter	*SPP*	Aerobic
Leuconostoc	*carnosum*	Aerobic
Leuconostoc	*mesenteroides*	Aerobic
Leuconostoc	*citreum*	Aerobic
Leuconostoc	*pseudomesenteroides*	Aerobic
Leuconostoc	*Leuconostoc*	Aerobic
Leuconostoc	*SPP*	Facultative Anaerobic
Leuconostoc	*inhae*	Facultative Anaerobic
Limnobacter	*thiooxidans*	Aerobic
Listeria	*grayi*	Facultative Anaerobic
Listeria	*monocytogenes*	Other
LLKB_g		Other
Loktanella	*atrilutea*	Aerobic
Lolium perenne	*Lolium perenne*	Other
Luteibacter	*SPP*	Aerobic
Luteibacter	*yeojuensis*	Aerobic
Luteimonas	*SPP*	Aerobic
Luteimonas	*huabeiensis*	Aerobic
Luteimonas	*tolerans*	Aerobic
Luteimonas	*terricola*	Aerobic
Luteimonas	*Mitacek01*	Other
Luteitalea	*SPP*	Aerobic
Luteococcus	*sediminum*	Aerobic
Luteolibacter	*SPP*	Aerobic
Luteolibacter	*yonseiensis*	Aerobic
Luteolibacter	*pohnpeiensis*	Aerobic
Lysinibacillus	*SPP*	Aerobic
Lysobacter	*SPP*	Aerobic
Lysobacter	*gummosus*	Aerobic
Lysobacter	*panaciterrae*	Aerobic
Lysobacter	*ginsengisoli*	Aerobic
Lysobacter	*humi*	Aerobic
Macadamia integrifolia	*Macadamia integrifolia*	Other
Macrochaete	*psychrophila*	Facultative Anaerobic

Macrococcus	*lamae*	Aerobic
Macrococcus	*equipercicus*	Aerobic
Macrococcus	*SPP*	Facultative Anaerobic
Malikia	*SPP*	Aerobic
Maribacter	*SPP*	Aerobic
Marinilactibacillus	*SPP*	Facultative Anaerobic
Marinobacter	*SPP*	Aerobic
Marinobacter	*maritimus*	Facultative Anaerobic
Marinomonas	*SPP*	Aerobic
Marinomonas	*arenicola*	Aerobic
Marmoricola	*SPP*	Aerobic
Marmoricola	*bigeumensis*	Aerobic
Marmoricola	*pocheonensis*	Aerobic
Marmoricola	*scoriae*	Aerobic
Massilia	*timonae*	Aerobic
Massilia	*SPP*	Aerobic
Massilia	*alkalitolerans*	Aerobic
Massilia	*aurea*	Aerobic
Massilia	*suwonensis*	Aerobic
Massilia	*brevitalea*	Aerobic
Massilia	*Telluria mixta*	Other
Medicago	*truncatula (barrel medic)*	Other
Megamonas	*SPP*	Anaerobic
Megasphaera	*SPP*	Anaerobic
Megasphaera	*massiliensis*	Anaerobic
Meiothermus	*SPP*	Aerobic
Mesorhizobium	*SPP*	Aerobic
Mesorhizobium	*jarvisii*	Aerobic
Mesorhizobium	*loti*	Aerobic
metagenome	*SPP*	Other
Methanobrevibacter	*smithii*	Anaerobic
Methylibium	*petroleiphilum*	Facultative Anaerobic
Methylobacillus	*SPP*	Facultative Anaerobic
Methylobacterium	*SPP*	Aerobic
Methylobacterium	*haplocladii*	Aerobic
Methylobacterium	*aminovorans*	Aerobic
Methylobacterium	*salsuginis*	Aerobic
Methylobacterium	*goesingense*	Aerobic
Methylobacterium	*mesophilicum*	Aerobic
Methylobacterium	*organophilum*	Aerobic
Methylobacterium	*persicinum*	Aerobic
Methylobacterium	*radiotolerans*	Aerobic
Methylobacterium	*thiocyanatum*	Aerobic
Methyloceanibacter	*caenitepidi*	Aerobic
Methylocella	*SPP*	Aerobic
Methylophilus	*SPP*	Aerobic
Methylophilus	*methylotrophus*	Aerobic
Methylopila	*SPP*	Aerobic

Methylopila	*oligotropha*	Aerobic
Methylorosula	*SPP*	Aerobic
Methyloversatilis	*thermotolerans*	Aerobic
Methyloversatilis	*universalis*	Aerobic
Methyloversatilis	*discipulorum*	Facultative Anaerobic
Methyloversatilis	*SPP*	Other
Micavibrio	*aeruginosavorus ARL-13*	Other
Microbacterium	*SPP*	Aerobic
Microbacterium	*aurum*	Aerobic
Microbacterium	*thalassium*	Aerobic
Microbacterium	*phyllosphaerae*	Aerobic
Microbacterium	*populi*	Aerobic
Microbacterium	*murale*	Aerobic
Microbacterium	*hydrocarbonoxydans*	Aerobic
Microbacterium	*foliorum*	Aerobic
Microbacterium	*hatanonis*	Aerobic
Microbacterium	*lacticum*	Aerobic
Microbacterium	*oxydans*	Aerobic
Microbacterium	*paludicola*	Aerobic
Microbacterium	*pygmaeum*	Aerobic
Microbacterium	*testaceum*	Aerobic
Microbacterium	*ginsengisoli*	Aerobic
Microbacterium	*lacus*	Aerobic
Microbacterium	*paraoxydans*	Aerobic
Microbacterium	*schleiferi*	Facultative Anaerobic
Microbacterium	*terregens*	Facultative Anaerobic
Microbacterium	*trichothecenolyticum*	Other
Micrococcus	*aloeverae*	Aerobic
Micrococcus	*luteus*	Aerobic
Micrococcus	*endophyticus*	Aerobic
Micrococcus	*cohnii*	Aerobic
Micrococcus	*flavus*	Aerobic
Micrococcus	*terreus*	Aerobic
Micrococcus	*SPP*	Other
Micrococcus	*lylae*	Facultative Anaerobic
Micrococcus	*antarcticus*	Facultative Anaerobic
Microcystis	*SPP*	Other
Microlunatus	*SPP*	Aerobic
Microlunatus	*phosphovorus*	Aerobic
Microlunatus	*panaciterrae*	Aerobic
Micromonospora	*pattaloongensis*	Aerobic
Micropruina	*SPP*	Facultative Anaerobic
Micropruina	*glycogenica*	Facultative Anaerobic
Microterricola	*viridarii*	Aerobic
Microvirga	*SPP*	Aerobic
Microvirga	*lotononidis*	Aerobic
Microvirga	*zambiensis*	Aerobic
Millettia pinnata	*Millettia pinnata*	Other

Millisia	*brevis*	Aerobic
Mitsuokella	*SPP*	Anaerobic
Mobilicoccus	*pelagius*	Facultative Anaerobic
Mobiluncus	*curtisii*	Anaerobic
Mobiluncus	*SPP*	Anaerobic
Modestobacter	*actinobacterium*	Aerobic
Modestobacter	*SPP*	Aerobic
Modestobacter	*lapidis*	Aerobic
Modestobacter	*versicolor*	Aerobic
Modestobacter	*roseus*	Aerobic
Mogibacterium	*timidum*	Anaerobic
Mogibacterium	*SPP*	Anaerobic
Mogibacterium	*diversum*	Anaerobic
Mogibacterium	*Ileibacterium massiliense*	Other
Moheibacter	*sediminis*	Aerobic
Moraxella	*SPP*	Aerobic
Moraxella	*atlantae*	Aerobic
Moraxella	*lincolnii*	Aerobic
Moraxella	*cuniculi*	Aerobic
Moraxella	*catarrhalis*	Aerobic
Moraxella	*nonliquefaciens*	Aerobic
Moraxella	*osloensis*	Aerobic
Moryella	*SPP*	Anaerobic
Mucilaginibacter	*SPP*	Facultative Anaerobic
Mucilaginibacter	*frigoritolerans*	Facultative Anaerobic
Murdochiella	*SPP*	Anaerobic
Murdochiella	*massiliensis*	Anaerobic
Murdochiella	*asaccharolytica*	Anaerobic
Murdochiella	*Levyella massiliensis*	Other
Muricoccus	*Roseomonas rosea*	Other
Musa	*itinerans*	Other
Mycobacterium	*SPP*	Aerobic
Mycobacterium	*gordonae*	Aerobic
Mycobacterium	*buckleii*	Aerobic
Mycobacterium	*hodleri*	Aerobic
Mycobacterium	*neoaurum*	Aerobic
Mycobacterium	*phocaicum*	Anaerobic
Mycoplasma	*SPP*	Anaerobic
Mycoplasma	*Chlamydia abortus*	Other
Myrmecia	*israelensis*	Other
Myroides	*SPP*	Aerobic
Myroides	*phaeus*	Aerobic
Nakamurella	*SPP*	Aerobic
Nakamurella	*multipartita*	Aerobic
Nakamurella	*flavida*	Aerobic
Nakamurella	*lactea*	Aerobic
Nakamurella	*panacisegetis*	Aerobic
Nannocystis	*SPP*	Aerobic

Naumannella	*halotolerans*	Aerobic
Naumannella	*SPP*	Aerobic
Negativicoccus	*succinicivorans*	Anaerobic
Negativicoccus	*Tissierellia bacterium S5-A11*	Other
Neisseria	*weaveri*	Aerobic
Neisseria	*bacilliformis*	Aerobic
Neisseria	*meningitidis*	Aerobic
Neisseria	*gonorrhoeae*	Other
Neisseria	*elongata*	Anaerobic
Neisseria	*polysaccharea*	Anaerobic
Neisseria	*SPP*	Other
Neisseria	*flavescens*	Facultative Anaerobic
Neisseria	*oralis*	Facultative Anaerobic
Neisseria	*sicca*	Facultative Anaerobic
Neisseria	*SPP Kingella sp.*	Other
Neisseria	*mucosa*	Other
Neomicrococcus	*Micrococcus lylae*	Other
Nesterenkonia	*SPP*	Aerobic
Nesterenkonia	*lacusekhoensis*	Aerobic
Nesterenkonia	*sandarakina*	Aerobic
Nevskia	*ramosa*	Aerobic
Nibribacter	*Rufibacter glacialis*	Other
Nicoletella	*semolina*	Anaerobic
Nicotiana	*tabacum (common tobacco)*	Other
Nitriliruptor	*SPP*	Aerobic
Nitrospira	*SPP*	Aerobic
Niveispirillum	*cyanobacteriorum*	Aerobic
Niveispirillum	*SPP Rhodocista sp.*	Other
Nocardia	*mikamii*	Aerobic
Nocardioides	*SPP*	Aerobic
Nocardioides	*bigeumensis*	Aerobic
Nocardioides	*insulae*	Aerobic
Nocardioides	*alpinus*	Aerobic
Nocardioides	*aestuarii*	Aerobic
Nocardioides	*szechwanensis*	Aerobic
Nocardioides	*jensenii*	Aerobic
Nocardioides	*glacieisoli*	Aerobic
Nocardioides	*iriomotensis*	Aerobic
Nocardioides	*lianchengensis*	Aerobic
Nocardioides	*oleivorans*	Aerobic
Nocardioides	*paucivorans*	Aerobic
Nocardioides	*plantarum*	Aerobic
Nocardioides	*terrigena*	Aerobic
Nocardioides	*aquaticus*	Aerobic
Nocardioides	*flavus*	Aerobic
Nocardiopsis	*SPP*	Aerobic
Nodosilinea	*SPP*	Aerobic

Nodosilinea PCC-7104	*Phormidium tenue NIES-30*	Other
Nodosilinea PCC-7104	*SPP Oscillatoriales cyanobacterium*	Other
Nodularia PCC-9350	*Aphanizomenon sp. NH-5*	Other
Nosocomiicoccus	*SPP*	Aerobic
Nosocomiicoccus	*ampullae*	Aerobic
Nostoc	*SPP*	Facultative Anaerobic
Novibacillus	*thermophilus*	Facultative Anaerobic
Noviherbaspirillum	*SPP*	Aerobic
Noviherbaspirillum	*Alligator sinensis (Chinese alligator)*	Other
Novosphingobium	*SPP*	Aerobic
Novosphingobium	*lentum*	Aerobic
Novosphingobium	*subterraneum*	Aerobic
Novosphingobium	*endophyticum*	Aerobic
Nyholmiella gymnostoma	*Nyholmiella gymnostoma*	Other
Nymphoides peltata	*Nymphoides peltata*	Other
Oceanobacillus	*arenosus*	Aerobic
Oceanobacillus	*profundus*	Aerobic
Oceanobacillus	*SPP*	Other
Ochrobactrum	*SPP*	Aerobic
Ochrobactrum	*anthropi*	Aerobic
Ochrobactrum	*intermedium*	Other
Odoribacter	*splanchnicus*	Anaerobic
Oerskovia	*SPP*	Facultative Anaerobic
Okibacterium	*fritillariae*	Aerobic
OLB13	*SPP Chloroflexi bacterium*	Other
OLB15	*Chloroflexi bacterium OLB15*	Other
OLB17	*SPP Acidobacteriaceae bacterium*	Other
Oligella	*urethralis*	Aerobic
Oligoflexus	*tunisiensis*	Aerobic
Oligoflexus	*SPP*	Aerobic
Oligoflexus	*SPP Desulfocurvus sp.*	Other
Olivibacter	*ginsengisoli*	Aerobic
Olsenella	*SPP*	Anaerobic
Olsenella	*Marseille*	Anaerobic
Olsenella	*uli*	Anaerobic
OM43 clade	*SPP*	Other
Oribacterium	*SPP*	Anaerobic
Oribacterium	*asaccharolyticum*	Anaerobic
Ornithinicoccus	*SPP*	Aerobic
Ornithinimicrobium	*SPP*	Aerobic
Ornithinimicrobium	*murale*	Aerobic
Ornithinimicrobium	*tianjinense*	Aerobic
Orobanche pancicii	*Orobanche pancicii*	Other
Oryza meyeriana	*Oryza meyeriana*	Other

Oscillatoria	*nigro-viridis*	Facultative Anaerobic
Oscillatoria	*SPP*	Facultative Anaerobic
Oscillibacter	*SPP*	Anaerobic
Ottowia	*beijingensis*	Aerobic
p-1088-a5 gut group	*SPP*	Other
Paenarthrobacter	*SPP*	Aerobic
Paenarthrobacter	*ilicis*	Aerobic
Paenibacillus	*lactis*	Facultative Anaerobic
Paenibacillus	*SPP*	Facultative Anaerobic
Paenibacillus	*castaneae*	Facultative Anaerobic
Paenibacillus	*typhae*	Facultative Anaerobic
Paenibacillus	*frigoriresistens*	Facultative Anaerobic
Paenibacillus	*pasadenensis*	Facultative Anaerobic
Paeniglutamicibacter	*Arthrobacter sp. SH-43B*	Other
Paeniglutamicibacter	*SPP*	Other
Paenisporosarcina	*quisquiliarum*	Aerobic
Paenochrobactrum	*glaciei*	Aerobic
Paenochrobactrum	*SPP*	Aerobic
Paludibacter	*SPP*	Anaerobic
Paludicola	*SPP*	Anaerobic
Panacagrimonas	*SPP*	Aerobic
Pannonibacter	*phragmitetus*	Facultative Anaerobic
Pantoea	*SPP*	Facultative Anaerobic
Pantoea	*eucrina*	Facultative Anaerobic
Pantoea	*agglomerans*	Facultative Anaerobic
Pantoea	*septica*	Facultative Anaerobic
Pantoea	*ananatis*	Facultative Anaerobic
Pantoea	*calida*	Facultative Anaerobic
Pantoea	*Enterobacter hormaechei*	Other
Papillibacter	*SPP*	Anaerobic
Parabacteroides	*SPP*	Anaerobic
Parabacteroides	*distasonis*	Anaerobic
Parabacteroides	*merdae*	Anaerobic
Paraburkholderia	*tropica*	Anaerobic
Paracoccus	*solventivorans*	Aerobic
Paracoccus	*yeei*	Aerobic
Paracoccus	*denitrificans*	Aerobic
Paracoccus	*contaminans*	Aerobic
Paracoccus	*alcaliphilus*	Aerobic
Paracoccus	*pacificus*	Aerobic
Paracoccus	*marcusii*	Aerobic
Paracoccus	*marinus*	Aerobic
Paracoccus	*sphaerophysae*	Aerobic
Paracoccus	*SPP*	Other
Paracoccus	*sanguinis*	Facultative Anaerobic
Paracoccus	*panacisoli*	Facultative Anaerobic
Paracoccus	*caeni*	Other
Paracoccus	*siganidrum*	Other

Paradevosia	*shaoguanensis*	Aerobic
Parafilimonas	*SPP*	Aerobic
Parafrigoribacterium	*SPP*	Aerobic
Paraoerskovia	*SPP*	Facultative Anaerobic
Paraprevotella	*SPP*	Anaerobic
Parascardovia	*denticolens*	Anaerobic
Parasegetibacter	*SPP*	Aerobic
Pardosa	*pseudoannulata*	Other
Parvimonas	*SPP*	Anaerobic
Parvimonas	*micra*	Anaerobic
Parvimonas	*Tissierellia bacterium KA00581*	Other
Parviterribacter	*SPP*	Aerobic
Pasteurella	*multocida*	Facultative Anaerobic
Pasteurella	*bettyae*	Facultative Anaerobic
Pasteurella	*Rodentibacter pneumotropicus*	Other
Pasteurellaceae	*SPP*	Facultative Anaerobic
Patulibacter	*minatonensis*	Aerobic
Paucibacter	*SPP*	Aerobic
Paucibacter	*oligotrophus*	Facultative Anaerobic
Pectobacterium	*carotovorum*	Facultative Anaerobic
Pediococcus	*pentosaceus*	Facultative Anaerobic
Pediococcus	*acidilactici*	Facultative Anaerobic
Pedobacter	*SPP*	Aerobic
Pedobacter	*insulae*	Aerobic
Pedobacter	*heparinus*	Aerobic
Pedobacter	*oryzae*	Aerobic
Pedobacter	*alluvionis*	Aerobic
Pedobacter	*koreensis*	Aerobic
Pedobacter	*agri*	Aerobic
Pedobacter	*caeni*	Aerobic
Pedomicrobium	*SPP*	Aerobic
Pelagibius	*SPP*	Aerobic
Pelomonas	*SPP*	Aerobic
Pelomonas	*saccharophila*	Aerobic
Penicillium	*chrysogenum*	Other
Penicillium	*expansum*	Other
Peptococcus	*SPP*	Anaerobic
Peptoniphilus	*harei*	Anaerobic
Peptoniphilus	*coxii*	Anaerobic
Peptoniphilus	*indolicus*	Anaerobic
Peptoniphilus	*SPP*	Anaerobic
Peptoniphilus	*timonensis*	Anaerobic
Peptoniphilus	*rhinitidis*	Anaerobic
Peptoniphilus	*phoceensis*	Anaerobic
Peptoniphilus	*obesi*	Anaerobic
Peptoniphilus	*lacrimalis*	Anaerobic

Peptoniphilus	*gorbachii*	Anaerobic
Peptoniphilus	*grossensis*	Anaerobic
Peptoniphilus	*senegalensis*	Anaerobic
Peptoniphilus	*tyrrelliae*	Facultative Anaerobic
Peptoniphilus	*urinimassiliensis*	Other
Peptostreptococcus	*SPP*	Anaerobic
Peptostreptococcus	*anaerobius*	Anaerobic
Peptostreptococcus	*stomatis*	Anaerobic
Peredibacter	*SPP delta proteobacterium*	Other
Peredibacter	*microbial mat SPP*	Other
Peredibacter	*SPP*	Other
Peredibacter	*Peredibacter starrii*	Other
Peredibacter	*Bacteriovorax sp. EPA*	Other
Perlucidibaca	*SPP*	Facultative Anaerobic
Persicaria	*minor*	Other
Persicitalea	*SPP*	Aerobic
Phascolarctobacterium	*SPP*	Anaerobic
Phaselicystis	*SPP delta proteobacterium*	Aerobic
Phaselicystis	*SPP*	Aerobic
Phenylobacterium	*SPP*	Facultative Anaerobic
Phormidesmis	*priestleyi*	Facultative Anaerobic
Phormidesmis	*SPP*	Facultative Anaerobic
Phormidium	*SPP*	Facultative Anaerobic
Photobacterium	*SPP*	Facultative Anaerobic
Photobacterium	*phosphoreum*	Facultative Anaerobic
Phreatobacter	*SPP*	Aerobic
Phycicoccus	*endophyticus*	Aerobic
Phycicoccus	*soli*	Aerobic
Phycicoccus	*bigeumensis*	Aerobic
Phyllobacterium	*SPP*	Aerobic
Phyllobacterium	*trifolii*	Aerobic
Physcomitrella patens	*Physcomitrella patens*	Other
Phytohabitans	*SPP*	Aerobic
Picea	*glauca (white spruce)*	Other
Pinus	*canariensis*	Other
Pinus	*greggii*	Other
Pinus	*morrisonicola*	Other
Pir4 lineage	*SPP*	Other
Piscicoccus	*intestinalis*	Facultative Anaerobic
Planctopirus	*limnophila*	Aerobic
Planctopirus	*limnophila DSM 3776*	Other
Planococcus	*SPP*	Aerobic
Planococcus	*kocurii*	Aerobic
Planococcus	*rifietoensis*	Aerobic
Planococcus	*massiliensis*	Aerobic
Planomicrobium	*SPP*	Aerobic
Planomicrobium	*okeanokoites*	Aerobic
Planomicrobium	*Planococcus massiliensis*	Other

Plant	*Canella*	Other
Plantibacter	*SPP*	Aerobic
Pleurocapsa	*minor*	Other
PMMR1	*SPP*	Other
Polaromonas	*SPP*	Aerobic
Polyangium	*brachysporum*	Aerobic
Polyangium	*SPP*	Other
Polymorphobacter	*SPP*	Aerobic
Polynucleobacter	*SPP*	Facultative Anaerobic
Polynucleobacter	*difficilis*	Facultative Anaerobic
Pontibacter	*SPP*	Aerobic
Populus tremula	*Populus tremula*	Other
Porphyrobacter	*donghaensis*	Aerobic
Porphyrobacter	*tepidarius*	Aerobic
Porphyromonas	*SPP*	Anaerobic
Porphyromonas	*endodontalis*	Anaerobic
Porphyromonas	*cangingivalis*	Anaerobic
Porphyromonas	*gingivalis*	Anaerobic
Porphyromonas	*gingivicanis*	Anaerobic
Porphyromonas	*crevioricanis*	Anaerobic
Porphyromonas	*bennonis*	Anaerobic
Porphyromonas	*canoris*	Anaerobic
Porphyromonas	*catoniae*	Anaerobic
Porphyromonas	*pasteri*	Anaerobic
Porphyromonas	*asaccharolytica*	Anaerobic
Porphyromonas	*circumdentaria*	Anaerobic
Porphyromonas	*somerae*	Anaerobic
Porphyromonas	*SPP Streptococcus sp.*	Other
Povalibacter	*SPP*	Aerobic
Prevotella	*timonensis*	Anaerobic
Prevotella	*melaninogenica*	Anaerobic
Prevotella	*disiens*	Anaerobic
Prevotella	*amnii*	Anaerobic
Prevotella	*oris*	Anaerobic
Prevotella	*SPP*	Anaerobic
Prevotella	*bergensis*	Anaerobic
Prevotella	*bivia*	Anaerobic
Prevotella	*intermedia*	Anaerobic
Prevotella	*aurantiaca*	Anaerobic
Prevotella	*oralis*	Anaerobic
Prevotella	*denticola*	Anaerobic
Prevotella	*veroralis*	Anaerobic
Prevotella	*buccae*	Anaerobic
Prevotella	*buccalis*	Anaerobic
Prevotella	*copri*	Anaerobic
Prevotella	*corporis*	Anaerobic
Prevotella	*histicola*	Anaerobic
Prevotella	*jejuni*	Anaerobic

Prevotella	*loescheii*	Anaerobic
Prevotella	*nanceiensis*	Anaerobic
Prevotella	*pallens*	Anaerobic
Prevotella	*albensis*	Anaerobic
Prevotella	*salivae*	Anaerobic
Prevotella	*Chlamydia trachomatis*	Other
Prevotellaceae	*SPP*	Anaerobic
Prevotellamassilia	*timonensis*	Anaerobic
Promicromonospora	*kroppenstedtii*	Aerobic
Propionibacterium	*namnetense*	Anaerobic
Propionibacterium	*acnes*	Facultative Anaerobic
Propionibacterium	*granulosum*	Facultative Anaerobic
Propionibacterium	*SPP*	Facultative Anaerobic
Propionibacterium	*propionicum*	Facultative Anaerobic
Propionibacterium	*acidifaciens*	Facultative Anaerobic
Propionibacterium	*avidum*	Facultative Anaerobic
Propionibacterium	*freudenreichii*	Facultative Anaerobic
Propioniciclava	*SPP*	Facultative Anaerobic
Propionimicrobium	*lymphophilum*	Anaerobic
Propionimicrobium	*SPP*	Anaerobic
Proteus	*mirabilis*	Facultative Anaerobic
Proteus	*vulgaris*	Facultative Anaerobic
Proteus	*SPP*	Facultative Anaerobic
Providencia	*rettgeri*	Facultative Anaerobic
Prunus	*mume (Japanese apricot)*	Other
Pseudarthrobacter	*SPP*	Aerobic
Pseudarthrobacter	*scleromae*	Aerobic
Pseudarthrobacter	*oxydans*	Aerobic
Pseudarthrobacter	*phenanthrenivorans*	Aerobic
Pseudescherichia	*vulneris*	Aerobic
Pseudoalteromonas	*SPP*	Aerobic
Pseudoalteromonas	*paragorgicola*	Aerobic
Pseudoalteromonas	*arctica*	Aerobic
Pseudoalteromonas	*nigrifaciens*	Aerobic
Pseudobutyrivibrio	*SPP*	Anaerobic
Pseudochrobactrum	*Brucellaceae bacterium PAOSE175*	Other
Pseudoclavibacter	*SPP*	Aerobic
Pseudoclavibacter	*bifida*	Aerobic
Pseudoclavibacter	*helvolus*	Aerobic
Pseudoclavibacter	*alba*	Aerobic
Pseudoclavibacter	*faecalis*	Aerobic
Pseudoclavibacter	*Zimmermannella faecalis ATCC 13722*	Other
Pseudoclavibacter	*Zimmermannella alba NBRC 15616*	Other
Pseudoflavonifractor	*SPP*	Anaerobic
Pseudoglutamicibacter	*albus*	Aerobic

Pseudokineococcus	*SPP*	Aerobic
Pseudokineococcus	*Ornithinimicrobium sp. YIM KMY41*	Other
Pseudolabrys	*SPP*	Aerobic
Pseudolabrys	*SPP Bradyrhizobiaceae bacterium*	Other
Pseudomonas	*alcaliphila*	Aerobic
Pseudomonas	*taiwanensis*	Aerobic
Pseudomonas	*SPP*	Aerobic
Pseudomonas	*pseudoalcaligenes*	Aerobic
Pseudomonas	*psychrotolerans*	Aerobic
Pseudomonas	*putida*	Aerobic
Pseudomonas	*fluorescens*	Aerobic
Pseudomonas	*tolaasii*	Aerobic
Pseudomonas	*luteola*	Aerobic
Pseudomonas	*pelagia*	Aerobic
Pseudomonas	*moraviensis*	Aerobic
Pseudomonas	*syringae*	Aerobic
Pseudomonas	*fulva*	Aerobic
Pseudomonas	*amygdali*	Aerobic
Pseudomonas	*lutea*	Aerobic
Pseudomonas	*argentinensis*	Aerobic
Pseudomonas	*poae*	Aerobic
Pseudomonas	*alcaligenes*	Aerobic
Pseudomonas	*coleopterorum*	Aerobic
Pseudomonas	*fragi*	Aerobic
Pseudomonas	*japonica*	Aerobic
Pseudomonas	*mosselii*	Aerobic
Pseudomonas	*oryzihabitans*	Aerobic
Pseudomonas	*parafulva*	Aerobic
Pseudomonas	*peli*	Aerobic
Pseudomonas	*stutzeri*	Aerobic
Pseudomonas	*trivialis*	Aerobic
Pseudomonas	*balearica*	Aerobic
Pseudomonas	*indoloxydans*	Aerobic
Pseudomonas	*koreensis*	Aerobic
Pseudomonas	*meridiana*	Aerobic
Pseudomonas	*monteilii*	Aerobic
Pseudomonas	*punonensis*	Aerobic
Pseudomonas	*toyotomiensis*	Aerobic
Pseudomonas	*tremae*	Aerobic
Pseudomonas	*umsongensis*	Aerobic
Pseudomonas	*vancouverensis*	Aerobic
Pseudomonas	*veronii*	Aerobic
Pseudomonas	*weihenstephanensis*	Aerobic
Pseudomonas	*yamanorum*	Aerobic
Pseudomonas	*aeruginosa*	Facultative Anaerobic
Pseudomonas	*oleovorans*	Facultative Anaerobic

Pseudomonas	*chlororaphis*	Facultative Anaerobic
Pseudomonas	*Burkholderia sp. NFACC33-1*	Other
Pseudomonas	*Spumella-like flagellate JBM08*	Other
Pseudomonas	*Kluyvera intermedia*	Other
Pseudomonas	*Stenotrophomonas rhizophila*	Other
Pseudomonas	*Humulus lupulus var. lupulus*	Other
Pseudomonas	*Sargassum henslowianum*	Other
Pseudomonas	*Teleogryllus commodus*	Other
Pseudomuriella	*schumacherensis*	Other
Pseudonocardia	*SPP*	Anaerobic
Pseudophormidium	*SPP*	Other
Pseudopropionibacterium	*SPP*	Aerobic
Pseudopropionibacterium	*propionicum*	Facultative Anaerobic
Pseudorhodobacter	*wandonensis*	Aerobic
Pseudorhodobacter	*SPP*	Aerobic
Pseudorhodobacter	*Rhodobacter sp. BACL10 MAG-120910-bin24*	Other
Pseudorhodoferax	*SPP*	Aerobic
Pseudoxanthomonas	*SPP*	Aerobic
Pseudoxanthomonas	*ginsengisoli*	Aerobic
Pseudoxanthomonas	*suwonensis*	Aerobic
Pseudoxanthomonas	*taiwanensis*	Aerobic
Psychrobacillus	*SPP*	Aerobic
Psychrobacter	*SPP*	Aerobic
Psychrobacter	*glacincola*	Aerobic
Psychrobacter	*cibarius*	Aerobic
Psychrobacter	*frigidicola*	Aerobic
Psychrobacter	*immobilis*	Aerobic
Psychrobacter	*psychrophilus*	Aerobic
Psychrobacter	*cryohalolentis*	Aerobic
Psychrobacter	*fozii*	Aerobic
Psychrobacter	*okhotskensis*	Aerobic
Psychrobacter	*sanguinis*	Aerobic
Psychrobacter	*faecalis*	Aerobic
Psychrobacter	*glaciei*	Aerobic
Psychrobacter	*aquimaris*	Facultative Anaerobic
Psychroglaciecola	*SPP*	Aerobic
Psychromonas	*arctica*	Anaerobic
Psychromonas	*SPP*	Facultative Anaerobic
Psychrosinus	*fermentans*	Other
Puia	*dinghuensis*	Aerobic
Pythium ultimum	*Pythium ultimum*	Other
Qipengyuania	*SPP*	Facultative Anaerobic
Quadrisphaera	*SPP*	Aerobic

Quercus robur	*Quercus robur*	Other
Racomitrium	*lanuginosum*	Other
Rahnella	*SPP*	Facultative Anaerobic
Rahnella	*aquatilis*	Facultative Anaerobic
Ralstonia	*insidiosa*	Aerobic
Ralstonia	*pickettii*	Aerobic
Ramlibacter	*SPP*	Aerobic
Ramlibacter	*rhizophilus*	Aerobic
Raoultella	*ornithinolytica*	Facultative Anaerobic
Rathayibacter	*festucae*	Aerobic
Rathayibacter	*SPP*	Aerobic
Rathayibacter	*tritici*	Aerobic
RB41	*SPP*	Other
RB41	*SPP Acidobacteria bacterium*	Other
Reyranella	*SPP*	Anaerobic
Rheinheimera	*SPP*	Aerobic
Rheinheimera	*tilapiae*	Aerobic
Rheinheimera	*soli*	Facultative Anaerobic
Rheinheimera	*vulgaris*	Other
Rhizobacter	*SPP*	Aerobic
Rhizobacter	*fulvus*	Aerobic
Rhizobacter	*Methylibium sp. Zs46*	Other
Rhizobium	*etli*	Aerobic
Rhizobium	*SPP*	Aerobic
Rhizobium	*cauense*	Aerobic
Rhizobium	*rosettiformans*	Aerobic
Rhizobium	*soli*	Aerobic
Rhizobium	*tropici*	Aerobic
Rhizobium	*leguminosarum*	Aerobic
Rhizobium	*SPP*	Aerobic
Rhizobium	*leguminosarum*	Aerobic
Rhizorhapis	*SPP*	Aerobic
Rhodanobacter	*SPP*	Aerobic
Rhodobacter	*SPP*	Facultative Anaerobic
Rhodococcus	*fascians*	Aerobic
Rhodococcus	*SPP*	Aerobic
Rhodococcus	*cerastii*	Aerobic
Rhodococcus	*corynebacterioides*	Aerobic
Rhodococcus	*qingshengii*	Aerobic
Rhodococcus	*kroppenstedtii*	Aerobic
Rhodocytophaga	*SPP*	Aerobic
Rhodoferax	*SPP*	Facultative Anaerobic
Rhodopila	*SPP*	Facultative Anaerobic
Rhodoplanes	*SPP*	Facultative Anaerobic
Rickettsiella	*Proasellus assaforensis*	Other
Rickettsiella	*isopodorum*	Other
Riemerella	*columbipharyngis*	Facultative Anaerobic

Rikenellaceae	*SPP*	Anaerobic
Rivibacter	*subsaxonicus*	Aerobic
Robinsoniella	*SPP*	Anaerobic
Romboutsia	*timonensis*	Anaerobic
Romboutsia	*SPP*	Anaerobic
Roseburia	*SPP*	Anaerobic
Roseburia	*faecis*	Anaerobic
Roseburia	*intestinalis*	Anaerobic
Roseburia	*inulinivorans*	Anaerobic
Roseomonas	*SPP*	Aerobic
Roseomonas	*gilardii*	Aerobic
Roseomonas	*rubra*	Aerobic
Roseomonas	*cervicalis*	Aerobic
Roseomonas	*frigidaquae*	Aerobic
Roseomonas	*elaeocarpi*	Aerobic
Roseomonas	*aquatica*	Aerobic
Roseomonas	*mucosa*	Aerobic
Roseomonas	*vinacea*	Aerobic
Rothia	*marina*	Aerobic
Rothia	*SPP*	Other
Rothia	*dentocariosa*	Facultative Anaerobic
Rothia	*mucilaginosa*	Facultative Anaerobic
Rothia	*amarae*	Facultative Anaerobic
Rothia	*nasimurium*	Facultative Anaerobic
Rothia	*terrae*	Facultative Anaerobic
Rothia	*Mycobacterium abscessus subsp. bolletii*	Other
Rubellimicrobium	*SPP*	Aerobic
Rubellimicrobium	*mesophilum*	Aerobic
Rubellimicrobium	*roseum*	Aerobic
Rubritepida	*SPP*	Aerobic
Rubrivirga	*SPP*	Aerobic
Rubus hybrid cultivar	*Rubus hybrid cultivar*	Other
Rudanella	*SPP*	Aerobic
Rufibacter	*SPP*	Aerobic
Rumex acetosa (garden sorrel)	*Rumex acetosa (garden sorrel)*	Other
Ruminiclostridium	*SPP*	Anaerobic
Ruminiclostridium	*siraeum*	Anaerobic
Ruminococcaceae	*SPP*	Anaerobic
Ruminococcus	*bromii*	Anaerobic
Ruminococcus	*SPP*	Anaerobic
Ruminococcus	*callidus*	Anaerobic
Ruminococcus	*gnavus*	Anaerobic
Ruminococcus	*lactaris*	Anaerobic
Ruminococcus	*torques*	Anaerobic
Ruminococcus	*SPP*	Anaerobic

Ruminofilibacter	*Ruminofilibacter xylanolyticum*	Other
Ruminofilibacter	*xylanolyticum*	Other
Runella	*SPP*	Aerobic
S31	*Staphylococcaceae bacterium S31*	Other
s3t2d-1089	*SPP Oxalobacteraceae bacterium*	Other
S5-A14a	*Clostridiales bacterium S5-A14a*	Other
Saccharibacillus	*sacchari*	Facultative Anaerobic
Saccharopolyspora	*rosea*	Aerobic
Saccharopolyspora	*qijiaojingensis*	Aerobic
Saccharopolyspora	*SPP*	Aerobic
Salinibacterium	*SPP*	Aerobic
Salinibacterium	*Rhodococcus sp. PIC-C4*	Other
Salinicoccus	*qingdaonensis*	Aerobic
Salinicoccus	*luteus*	Aerobic
Salinicoccus	*SPP*	Aerobic
Salinicoccus	*roseus*	Aerobic
Salinicoccus	*salitudinis*	Aerobic
Salinicola	*SPP*	Facultative Anaerobic
Salinimicrobium	*gaetbulicola*	Aerobic
Salix integra	*Salix integra*	Other
Salmonella	*enterica*	Facultative Anaerobic
Salvia pomifera	*Salvia pomifera*	Other
Sandaracinobacter	*SPP*	Aerobic
Sanguibacter	*inulinus*	Facultative Anaerobic
Sarcina	*SPP*	Anaerobic
Scardovia	*wiggsiae*	Anaerobic
Schlegelella	*SPP*	Aerobic
Schlegelella	*thermodepolymerans*	Aerobic
Sclerospora	*graminicola*	Other
Scytonema	*SPP*	Facultative Anaerobic
Scytonema UTEX 2349	*Toxopsis calypsus PLF*	Other
Sediminibacterium	*SPP*	Aerobic
Segetibacter	*SPP*	Aerobic
Selenomonas	*SPP*	Anaerobic
Selenomonas	*artemidis*	Anaerobic
Selenomonas	*sputigena*	Anaerobic
Sellimonas	*intestinalis*	Anaerobic
Senegalia	*massiliensis*	Anaerobic
Senna alexandrina	*Senna alexandrina*	Other
Serratia	*liquefaciens*	Aerobic
Serratia	*SPP*	Facultative Anaerobic
Serratia	*marcescens*	Facultative Anaerobic
Serratia	*symbiotica*	Facultative Anaerobic
Serratia	*Serratia*	Facultative Anaerobic

Serratia	*proteamaculans*	Facultative Anaerobic
Serratia	*fonticola*	Facultative Anaerobic
Serratia	*rubidaea*	Facultative Anaerobic
Serratia	*quinivorans*	Facultative Anaerobic
Serratia	*vespertilionis*	Facultative Anaerobic
Serratia	*Ewingella americana*	Other
Serratia	*Rahnella aquatilis*	Other
Serratia	*Hafnia sp.*	Other
Shewanella	*SPP*	Facultative Anaerobic
Shewanella	*hanedai*	Facultative Anaerobic
Shewanella	*xiamenensis*	Facultative Anaerobic
Shimwellia	*SPP*	Facultative Anaerobic
Shimwellia	*blattae*	Other
Shinella	*SPP*	Facultative Anaerobic
SH-PL14	*SPP planctomycete*	Other
SH-PL14	*SPP*	Other
Siccibacter	*SPP*	Facultative Anaerobic
Silanimonas	*SPP*	Aerobic
Simplicispira	*metamorpha*	Anaerobic
Singulisphaera	*SPP*	Aerobic
Sinobaca	*Marinococcus sp. GSP31*	Other
Siphonobacter	*SPP*	Aerobic
Skermanella	*SPP*	Facultative Anaerobic
SM1A02	*SPP*	Other
Sneathia	*amnii*	Anaerobic
Snodgrassella	*SPP*	Anaerobic
Soehngenia	*SPP*	Anaerobic
Solanum	*melongena (eggplant)*	Other
Solanum incanum	*Solanum incanum*	Other
Solibacillus	*silvestris*	Aerobic
Solirubrobacter	*SPP*	Aerobic
Solirubrobacterales bacterium 67-14	*SPP*	Aerobic
Solobacterium	*SPP*	Anaerobic
Sorangium	*SPP*	Other
Sphaerotilus	*SPP*	Aerobic
Sphingoaurantiacus	*SPP*	Aerobic
Sphingobacterium	*SPP*	Aerobic
Sphingobacterium	*multivorum*	Aerobic
Sphingobacterium	*daejeonense*	Aerobic
Sphingobacterium	*spiritivorum*	Aerobic
Sphingobacterium	*paucimobilis*	Aerobic
Sphingobacterium	*cladoniae*	Aerobic
Sphingobacterium	*kitahiroshimense*	Aerobic
Sphingobacterium	*lactis*	Aerobic
Sphingobacterium	*thermophilum*	Aerobic
Sphingobacterium	*Brassica oleracea var. capitata (cabbage)*	Other

Sphingobacterium	*Oryza sativa Indica Group (long-grained rice)*	Other
Sphingobium	*SPP*	Aerobic
Sphingobium	*qiguonii*	Aerobic
Sphingobium	*yanoikuyae*	Aerobic
Sphingomonas	*SPP*	Aerobic
Sphingomonas	*prati*	Aerobic
Sphingomonas	*roseiflava*	Aerobic
Sphingomonas	*naphthae*	Aerobic
Sphingomonas	*phyllosphaerae*	Aerobic
Sphingomonas	*parapaucimobilis*	Aerobic
Sphingomonas	*adhaesiva*	Aerobic
Sphingomonas	*mali*	Aerobic
Sphingomonas	*aerolata*	Aerobic
Sphingomonas	*alpina*	Aerobic
Sphingomonas	*arantia*	Aerobic
Sphingomonas	*desiccabilis*	Aerobic
Sphingomonas	*echinoides*	Aerobic
Sphingomonas	*fonticola*	Aerobic
Sphingomonas	*melonis*	Aerobic
Sphingomonas	*molluscorum*	Aerobic
Sphingomonas	*oligophenolica*	Aerobic
Sphingomonas	*vulcanisoli*	Aerobic
Sphingomonas	*Peromyscus californicus (California mouse)*	Other
Sphingomonas	*Caulobacter sp. HWE-A10*	Other
Sphingomonas	*bosoensis*	Other
Sphingopyxis	*alaskensis*	Aerobic
Sphingopyxis	*ginsengisoli*	Aerobic
Sphingorhabdus	*SPP*	Aerobic
Sphingorhabdus	*planktonica*	Aerobic
Sphingosinicella	*SPP*	Aerobic
Spirosoma	*SPP*	Aerobic
Spirosoma	*montaniterrae*	Aerobic
Spirosoma	*arcticum*	Aerobic
Spirosoma	*linguale*	Aerobic
Spirosoma	*radiotolerans*	Aerobic
Spirosoma	*rigui*	Facultative Anaerobic
Sporobacter	*SPP*	Anaerobic
Sporocytophaga	*myxococcoides*	Aerobic
Sporosarcina	*ureae*	Aerobic
Sporosarcina	*soli*	Aerobic
Sporosarcina	*luteola*	Anaerobic
Sporosarcina	*SPP*	Facultative Anaerobic
Staphylococcus	*schweitzeri*	Aerobic
Staphylococcus	*epidermidis*	Facultative Anaerobic
Staphylococcus	*aureus*	Facultative Anaerobic
Staphylococcus	*hominis*	Facultative Anaerobic

Staphylococcus	*caprae*	Facultative Anaerobic
Staphylococcus	*haemolyticus*	Facultative Anaerobic
Staphylococcus	*warneri*	Facultative Anaerobic
Staphylococcus	*xylosus*	Facultative Anaerobic
Staphylococcus	*saccharolyticus*	Facultative Anaerobic
Staphylococcus	*cohnii*	Facultative Anaerobic
Staphylococcus	*lentus*	Facultative Anaerobic
Staphylococcus	*condimenti*	Facultative Anaerobic
Staphylococcus	*lugdunensis*	Facultative Anaerobic
Staphylococcus	*simulans*	Facultative Anaerobic
Staphylococcus	*pasteuri*	Facultative Anaerobic
Staphylococcus	*saprophyticus*	Facultative Anaerobic
Staphylococcus	*pettenkoferi*	Facultative Anaerobic
Staphylococcus	*equorum*	Facultative Anaerobic
Staphylococcus	*SPP*	Facultative Anaerobic
Staphylococcus	*vitulinus*	Facultative Anaerobic
Staphylococcus	*kloosii*	Facultative Anaerobic
Staphylococcus	*gallinarum*	Facultative Anaerobic
Staphylococcus	*canus*	Facultative Anaerobic
Staphylococcus	*auricularis*	Facultative Anaerobic
Staphylococcus	*capitis*	Facultative Anaerobic
Staphylococcus	*nepalensis*	Facultative Anaerobic
Staphylococcus	*petrasii*	Facultative Anaerobic
Staphylococcus	*schleiferi*	Facultative Anaerobic
Staphylococcus	*simiae*	Facultative Anaerobic
Staphylococcus	*succinus*	Facultative Anaerobic
Starkeya	*koreensis*	Aerobic
Starkeya	*novella*	Aerobic
Stenotrophobacter	*SPP*	Aerobic
Stenotrophobacter	*terrae*	Aerobic
Stenotrophomonas	*maltophilia*	Aerobic
Stenotrophomonas	*SPP*	Aerobic
Stenotrophomonas	*rhizophila*	Aerobic
Stenotrophomonas	*chelatiphaga*	Aerobic
Stenotrophomonas	*pavanii*	Aerobic
Stenotrophomonas	*acidaminiphila*	Aerobic
Stenotrophomonas	*nitritireducens*	Aerobic
Stenotrophomonas	*Pseudomonas sp. MDFPXXVIIE320c*	Other
Steroidobacter	*SPP*	Aerobic
Stomatobaculum	*SPP*	Anaerobic
Streptobacillus	*SPP*	Facultative Anaerobic
Streptococcus	*anginosus*	Anaerobic
Streptococcus	*constellatus*	Anaerobic
Streptococcus	*cristatus*	Anaerobic
Streptococcus	*dysgalactiae*	Anaerobic
Streptococcus	*equinus*	Anaerobic
Streptococcus	*infantis*	Anaerobic

Streptococcus	*lactarius*	Anaerobic
Streptococcus	*oralis*	Anaerobic
Streptococcus	*peroris*	Anaerobic
Streptococcus	*pneumoniae*	Anaerobic
Streptococcus	*salivarius*	Facultative Anaerobic
Streptococcus	*thermophilus*	Facultative Anaerobic
Streptococcus	*parasanguinis*	Facultative Anaerobic
Streptococcus	*SPP*	Facultative Anaerobic
Streptococcus	*urinalis*	Facultative Anaerobic
Streptococcus	*pseudopneumoniae*	Facultative Anaerobic
Streptococcus	*pyogenes*	Facultative Anaerobic
Streptococcus	*dentisani*	Facultative Anaerobic
Streptococcus	*mutans*	Facultative Anaerobic
Streptococcus	*gordonii*	Facultative Anaerobic
Streptococcus	*sobrinus*	Facultative Anaerobic
Streptococcus	*sanguinis*	Facultative Anaerobic
Streptococcus	*agalactiae*	Facultative Anaerobic
Streptococcus	*mitis*	Facultative Anaerobic
Streptococcus	*sinensis*	Facultative Anaerobic
Streptococcus	*suis*	Facultative Anaerobic
Streptococcus	*equi*	Facultative Anaerobic
Streptococcus	*australis*	Facultative Anaerobic
Streptococcus	*rubneri*	Facultative Anaerobic
Streptococcus	*timonensis*	Facultative Anaerobic
Streptococcus	*broughtonii*	Other
Streptococcus sp. oral clone ASCB12	*SPP*	Facultative Anaerobic
Streptomyces	*panacagri*	Aerobic
Streptomyces	*albus*	Aerobic
Streptomyces	*pratensis*	Aerobic
Streptomyces	*canus*	Aerobic
Striga hermonthica	*Striga hermonthica*	Other
Subdoligranulum	*SPP*	Anaerobic
Subgroup 10	*SPP*	Other
Subtercola	*boreus*	Aerobic
Subtercola	*SPP*	Aerobic
Sulfurovum	*SPP*	Facultative Anaerobic
Sutterella	*SPP*	Anaerobic
Sutterella	*wadsworthensis*	Anaerobic
Sva0996 marine group	*SPP*	Other
Symbiochloris handae	*Symbiochloris handae*	Other
Syngonanthus chrysanthus	*Syngonanthus chrysanthus*	Other
Tabrizicola	*SPP*	Facultative Anaerobic
Tabrizicola	*Haematobacter sp. BC14248*	Other
Tahibacter	*SPP*	Aerobic
Taibaiella	*SPP*	Aerobic
Tannerella	*SPP*	Anaerobic

Tardiphaga	*SPP*	Aerobic
Taxus mairei	*Taxus mairei*	Other
Tepidimonas	*fonticaldi*	Aerobic
Tepidiphilus	*SPP*	Facultative Anaerobic
Tepidiphilus	*succinatimandens*	Facultative Anaerobic
Terrabacter	*SPP*	Aerobic
Terrabacter	*tumescens*	Aerobic
Terracoccus	*luteus*	Aerobic
Terriglobus	*aquaticus*	Aerobic
Terrimonas	*SPP*	Aerobic
Terrisporobacter	*SPP*	Anaerobic
Terrisporobacter	*sordellii*	Anaerobic
Terrisporobacter	*mayombei*	Anaerobic
Tessaracoccus	*SPP*	Other
Tessaracoccus	*flavescens*	Facultative Anaerobic
Tessaracoccus	*bendigoensis*	Facultative Anaerobic
Tessaracoccus	*rhinocerotis*	Facultative Anaerobic
Tetragenococcus	*halophilus*	Facultative Anaerobic
Tetrasphaera	*japonica*	Aerobic
Tetrasphaera	*SPP*	Aerobic
Tetrasphaera	*australiensis*	Aerobic
Tetrasphaera	*Sanguibacter sp. enrichment culture clone VanCtr9*	Other
Thauera	*SPP*	Facultative Anaerobic
Thauera	*phenylacetica*	Facultative Anaerobic
Thermicanus	*SPP*	Facultative Anaerobic
Thermoactinomyces	*SPP*	Aerobic
Thermoactinomyces	*vulgaris*	Aerobic
Thermoanaerobacterium	*saccharolyticum*	Anaerobic
Thermobacillus	*Xylanobacillus xylanolyticus*	Other
Thermomonas	*SPP*	Aerobic
Thermomonas	*carbonis*	Aerobic
Thermomonas	*haemolytica*	Aerobic
Thermomonas	*koreensis*	Aerobic
Thermus	*scotoductus*	Aerobic
Thermus	*SPP*	Aerobic
Thermus	*amyloliquefaciens*	Aerobic
Thiobacillus	*SPP*	Aerobic
Thiobacillus	*thiophilus*	Facultative Anaerobic
Thiomonas	*SPP*	Aerobic
Thuja	*standishii*	Other
Timonella	*Jonesia sp. Z4*	Other
Tissierella	*SPP*	Anaerobic
Tissierellia	*SPP*	Other
TM7 phylum sp. canine oral taxon 250	*TM7 phylum sp. canine oral taxon 250*	Other
TM7 phylum sp. oral clone FR058	*TM7 phylum sp. oral clone FR058*	Other

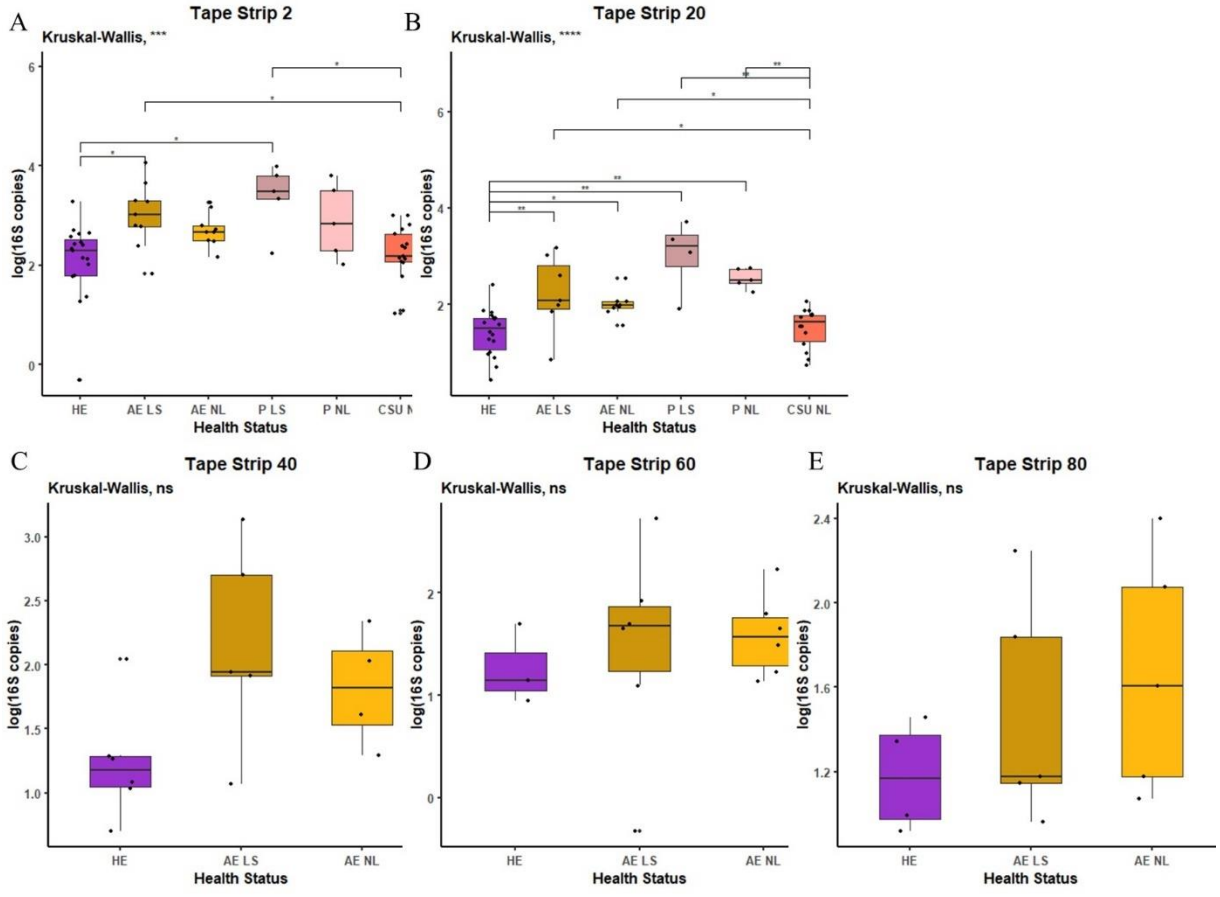
Tolumonas	*auensis*	Facultative Anaerobic
Treponema	*SPP*	Anaerobic
Treponema	*vincentii*	Anaerobic
Treponema	*refringens*	Anaerobic
Treponema	*socranskii*	Anaerobic
Trichococcus	*SPP*	Aerobic
Trichococcus	*patagoniensis*	Facultative Anaerobic
Triticum	*aestivum (bread wheat)*	Other
Truepera	*SPP*	Aerobic
Trueperella	*bernardiae*	Facultative Anaerobic
Tsukamurella	*inchonensis*	Aerobic
Turicella	*otitidis*	Anaerobic
Turicibacter	*SPP*	Anaerobic
Turicibacter	*sanguinis*	Anaerobic
Tychonema	*SPP*	Facultative Anaerobic
Uliginosibacterium	*SPP*	Aerobic
Uliginosibacterium	*paludis*	Aerobic
uncultured	*SPP*	Aerobic
uncultured	*Anaerovorax*	Anaerobic
uncultured	*flavefaciens*	Other
uncultured	*Photorhabdus luminescens*	Other
uncultured	*SPP cyanobacterium*	Other
uncultured	*SPP Clostridiisalibacter sp.*	Other
uncultured	*Clostridium phoceensis*	Other
uncultured	*Eubacterium*	Other
uncultured	*SPP compost bacterium*	Other
uncultured	*SPP Flavobacteriia bacterium*	Other
uncultured	*Tetraselmis sp. GSL018*	Other
uncultured	*SPP marine bacterium*	Other
uncultured	*SPP Chitinophagaceae bacterium*	Other
uncultured	*Taibaiella sp.*	Other
uncultured	*SPP Bacteroidetes bacterium*	Other
uncultured	*SPP alpha proteobacterium*	Other
uncultured	*SPP endolithic bacterium*	Other
uncultured	*Sphingomonas sp. PlS1*	Other
uncultured	*Roseomonas sp. OTB25*	Other
uncultured	*Pseudorhodobacter sp.*	Other
uncultured	*SPP Paracraurococcus sp.*	Other
uncultured	*SPP Sphingomonadaceae bacterium*	Other
uncultured	*Paracraurococcus sp. 1PNM-27*	Other
uncultured	*marine SPP*	Other
uncultured	*SPP Microbacteriaceae bacterium*	Other

uncultured	*Megophrys sangzhiensis*	Other
uncultured	*SPP Chloroflexus sp.*	Other
uncultured	*SPP Acidobacterium sp.*	Other
uncultured	*SPP soil bacterium*	Other
uncultured	*SPP Caldilinea sp.*	Other
uncultured	*SPP sludge bacterium A40*	Other
uncultured	*SPP Verrucomicrobium sp.*	Other
uncultured	*SPP Alcaligenes sp.*	Other
uncultured	*SPP proteobacterium*	Other
uncultured	*Brevundimonas sp. Tibet-IBa1*	Other
uncultured Acidobacteria bacterium	*SPP*	Other
uncultured Acidobacteria bacterium	*SPP Acidobacteria bacterium*	Other
uncultured actinobacterium	*SPP actinobacterium*	Other
uncultured actinobacterium		Other
uncultured alpha proteobacterium	*SPP alpha proteobacterium*	Other
uncultured Anaerolineae bacterium	*SPP Anaerolineae bacterium*	Other
uncultured bacterium		Aerobic
uncultured bacterium	*SPP*	Other
uncultured Bacteroidetes bacterium	*SPP Bacteroidetes bacterium*	Other
uncultured candidate division WS6 bacterium	*SPP candidate division WS6 bacterium*	Other
uncultured Candidatus Saccharibacteria bacterium	*SPP*	Other
uncultured Carnobacterium sp.	*SPP Carnobacterium sp.*	Other
uncultured Chlorophyta	*SPP Chlorophyta*	Other
uncultured compost bacterium	*SPP compost bacterium*	Other
uncultured Corynebacterium sp.	*SPP Corynebacterium sp.*	Other
uncultured cyanobacterium	*SPP cyanobacterium*	Other
uncultured diatom	*SPP diatom*	Other
uncultured endolithic bacterium	*SPP endolithic bacterium*	Other
uncultured eukaryote	*SPP eukaryote*	Other
uncultured Frankineae bacterium	*SPP Frankineae bacterium*	Other
uncultured gamma proteobacterium	*SPP gamma proteobacterium*	Other
uncultured Kribbella sp.	*SPP Kribbella sp.*	Other
uncultured organism	*SPP*	Other
uncultured phototrophic eukaryote	*SPP phototrophic eukaryote*	Other
uncultured Phyllobacteriaceae bacterium	*SPP Phyllobacteriaceae bacterium*	Other
uncultured Planctomycetales bacterium	*SPP Planctomycetales bacterium*	Other
uncultured planctomycete	*SPP planctomycete*	Other
uncultured prokaryote	*SPP prokaryote*	Other

uncultured Rubrobacteria bacterium	*SPP Rubrobacteria bacterium*	Other
uncultured rumen bacterium	*SPP rumen bacterium*	Other
uncultured sludge bacterium H46	*SPP sludge bacterium H46*	Other
uncultured soil bacterium	*SPP soil bacterium*	Other
uncultured Thermomicrobia bacterium	*SPP Thermomicrobia bacterium*	Other
Undibacterium	*oligocarboniphilum*	Aerobic
unidentified	*SPP*	Other
Ureaplasma	*parvum*	Facultative Anaerobic
Ureaplasma	*SPP*	Facultative Anaerobic
Ureibacillus	*SPP*	Aerobic
Ureibacillus	*suwonensis*	Aerobic
UTBCD1	*SPP*	Other
Vagococcus	*SPP*	Facultative Anaerobic
Varibaculum	*SPP*	Anaerobic
Variovorax	*paradoxus*	Aerobic
Variovorax	*SPP*	Other
Variovorax	*soli*	Facultative Anaerobic
Veillonella	*SPP*	Anaerobic
Veillonella	*seminalis*	Anaerobic
Veillonella	*atypica*	Anaerobic
Veillonella	*montpellierensis*	Anaerobic
Veillonella	*parvula*	Anaerobic
Verticia	*SPP*	Facultative Anaerobic
Vibrio	*SPP*	Facultative Anaerobic
Vibrio	*furnissii*	Facultative Anaerobic
Vibrio	*alginolyticus*	Facultative Anaerobic
Vibrio	*navarrensis*	Facultative Anaerobic
Vibrio	*fluvialis*	Facultative Anaerobic
Vibrio	*litoralis*	Facultative Anaerobic
Vicia	*faba (fava bean)*	Other
Virgibacillus	*SPP*	Facultative Anaerobic
Vischeria sp. CAUP Q 202	*Vischeria sp. CAUP Q 202*	Other
Viscum	*album*	Other
Vitreoscilla	*SPP*	Aerobic
Vogesella	*SPP*	Aerobic
Vulcaniibacterium	*SPP*	Aerobic
Vulcaniibacterium	*thermophilum*	Aerobic
Wautersiella	*falsenii*	Aerobic
Wautersiella	*SPP*	Aerobic
Weissella	*viridescens*	Anaerobic
Weissella	*confusa*	Anaerobic
Weissella	*soli*	Anaerobic
Williamsia	*SPP*	Aerobic
Williamsia	*maris*	Aerobic
Williamsia	*muralis*	Aerobic
Wohlfahrtiimonas	*larvae*	Facultative Anaerobic

Wolbachia	*pipientis*	Other
Xanthobacter	*autotrophicus*	Aerobic
Xanthobacter	*SPP*	Aerobic
Xanthomonas	*arboricola*	Aerobic
Xanthomonas	*campestris*	Aerobic
Xanthomonas	*axonopodis*	Aerobic
Xanthomonas	*vesicatoria*	Aerobic
Xanthomonas	*SPP*	Aerobic
Xylochloris irregularis	*Xylochloris irregularis*	Other
Xylophilus	*ampelinus*	Aerobic
Yaniella	*halotolerans*	Aerobic
Yersinia	*enterocolitica*	Facultative Anaerobic
Yimella	*Dermacoccus sp. BSi20643*	Other
Yokenella	*regensburgei*	Anaerobic
Yonghaparkia	*SPP*	Aerobic
Yonghaparkia	*Root332*	Aerobic
Zimmermannella	*faecalis*	Aerobic
Zimmermannella	*alba*	Aerobic
Ziziphus jujuba	*Ziziphus jujuba*	Other
Zoogloea	*SPP*	Aerobic

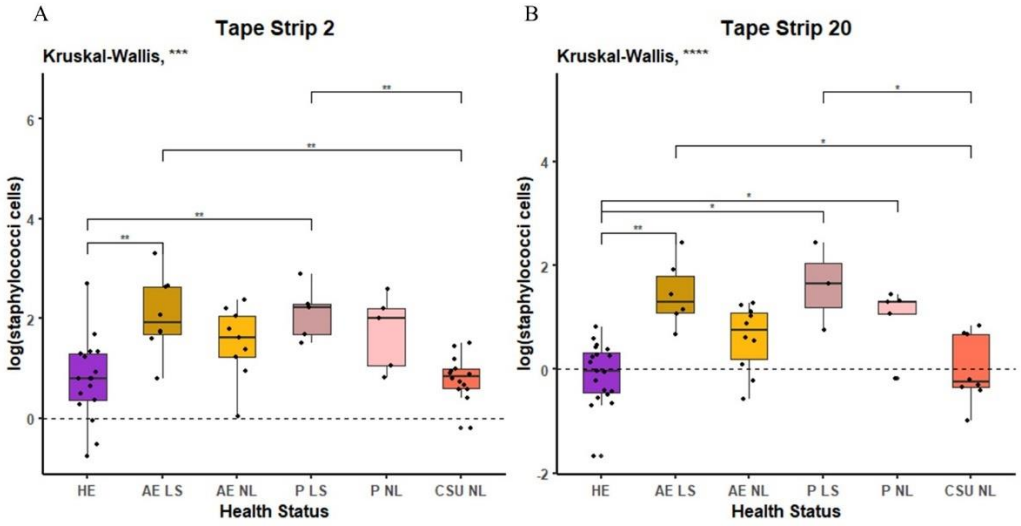
7.1.2 Section 3.1.1 Supplement



Supplement Figure 7.1 Abundance of Bacteria Across Skin Depth

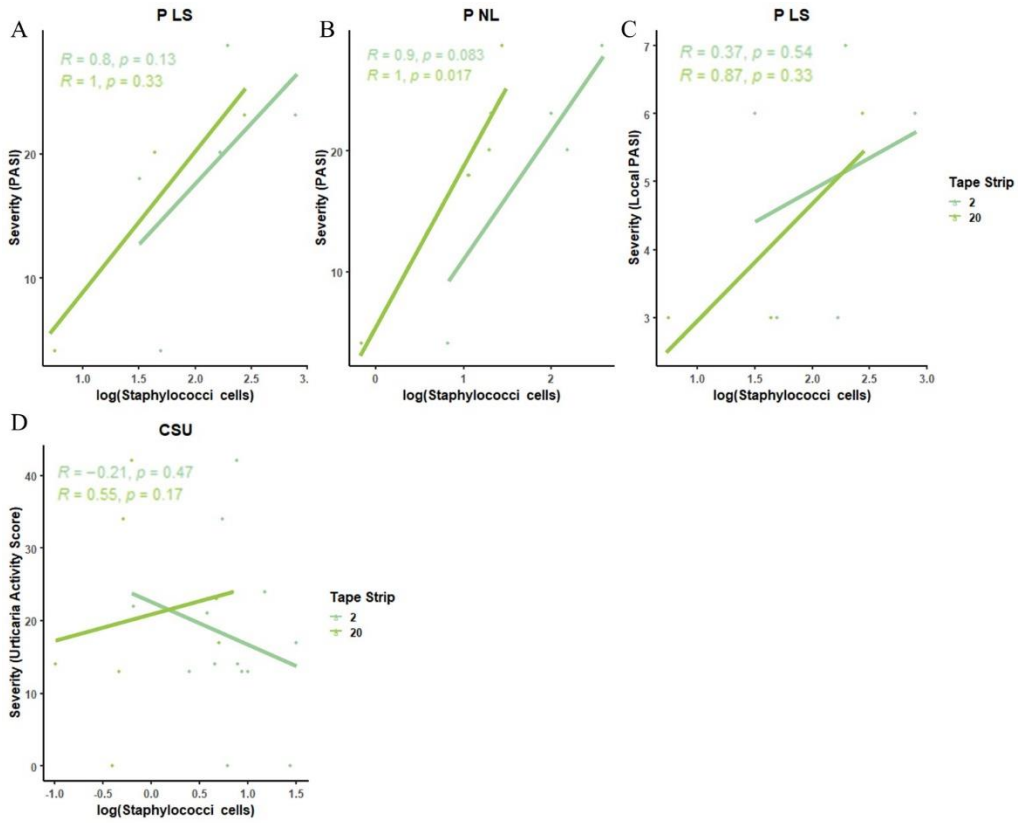
Boxplot with overlapped dot plot of the log(16S copies) according to health status and separated by tape strip (A) Tape strip 2. (B) Tape strip 20. (C) Tape strip 40. (D) Tape strip 60. (E) Tape strip 80. Samples with undetected 16S reads were removed and background 16S copies was subtracted. Post-hoc test was Dunn's test. Color scheme and Abbreviations: Psoriasis lesional (P LS; dark pink), Psoriasis non-lesional (P NL, light pink), Chronic Spontaneous Urticaria (CSU, coral), Atopic Eczema non-lesional (AE NL, yellow), Atopic Eczema lesional (AE LS, dark yellow), healthy (HE, purple).

7.1.3 Section 3.1.2 Supplement



Supplement Figure 7.2 Abundance of Staphylococci at Tape Strip 2 and 20

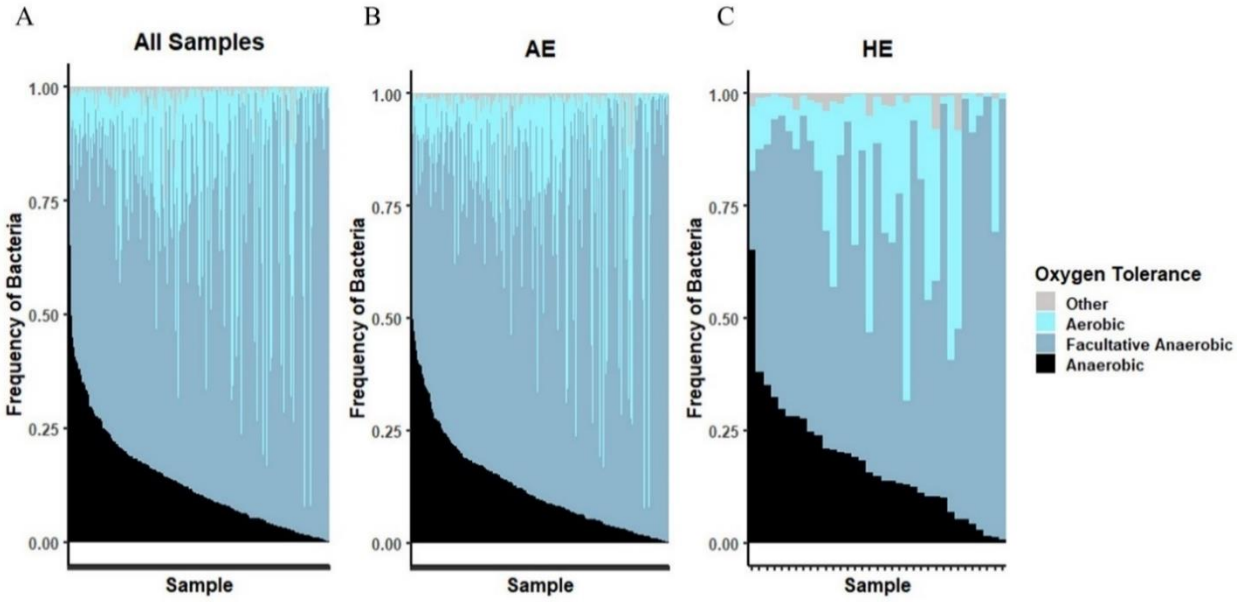
Boxplot with overlapped dot plot of the log(Staphylococci Cells) according to health status separated by tape strip depth (A) Tape strip 2. (B) Tape strip 20. Samples with undetected 16S reads and staphylococci cells were removed. Staphylococci cells are calculated according to TUF2 copy number, where there is one TUF2 copy per cell. Significance was determined by Kruskal-Wallis with post-hoc Dunn's test. Color scheme and Abbreviations: Psoriasis lesional (P LS; dark pink), Psoriasis non-lesional (P NL, light pink), Chronic Spontaneous Urticaria (CSU, coral), Atopic Eczema non-lesional (AE NL, yellow), Atopic Eczema lesional (AE LS, dark yellow), healthy (HE, purple).



Supplement Figure 7.3 Correlation of Severity to Staphylococci in Psoriasis and CSU

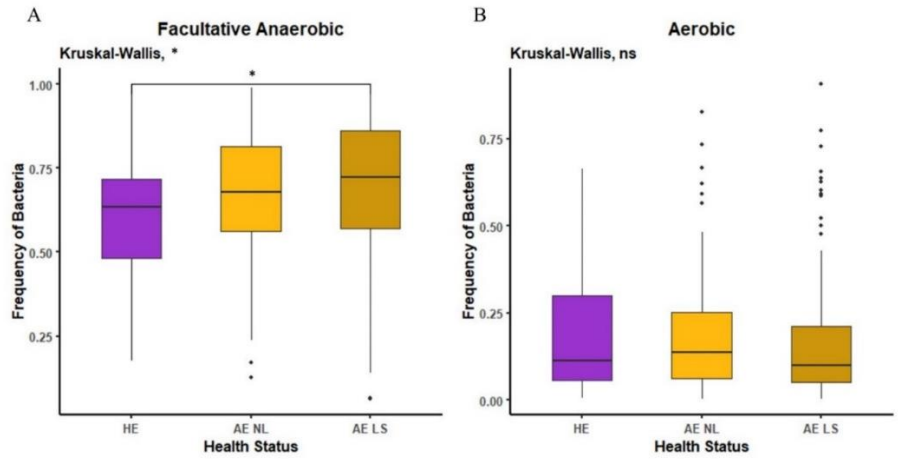
(A) P LS, PASI (B) P NL, PASI (C) P LS, local PASI (D) CSU, Urticaria Activity Score. All data was stratified by Tape Strip: TS2 (darkseagreen3), TS20 (darkolivegreen3), TS40 (darkolivegreen4), TS60 (darkolivegreen), TS80 (darkgreen). Abbreviations: Psoriasis lesional (P LS), Psoriasis non-lesional (P NL), Chronic Spontaneous Urticaria (CSU).

7.1.4 Section 3.2.2 Supplement



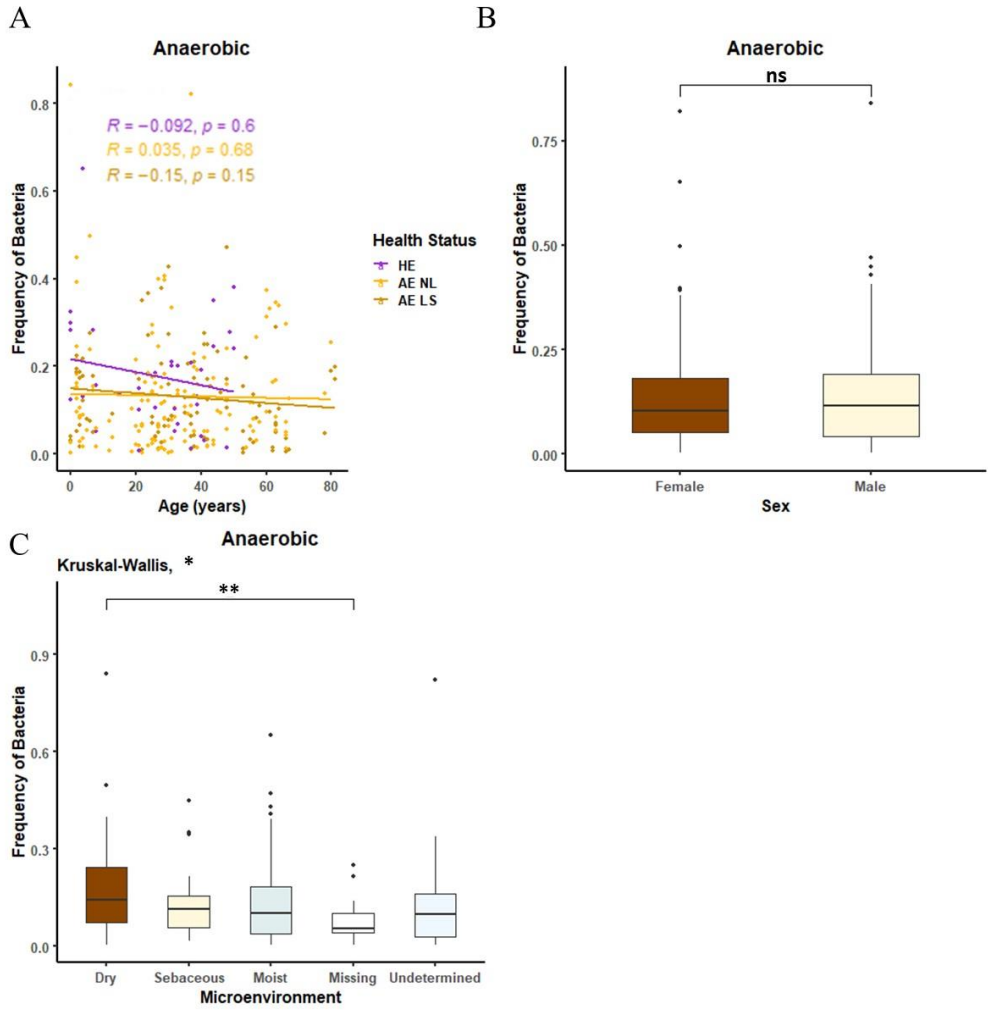
Supplement Figure 7.4 Frequency of OTUs per Sample Grouped by Oxygen Tolerance

(A) all samples (B) Atopic eczema (AE) samples (C) Healthy (HE) samples. Color scheme: other (grey), aerobic (bright blue), facultative anaerobic (dull blue), anaerobic (black).



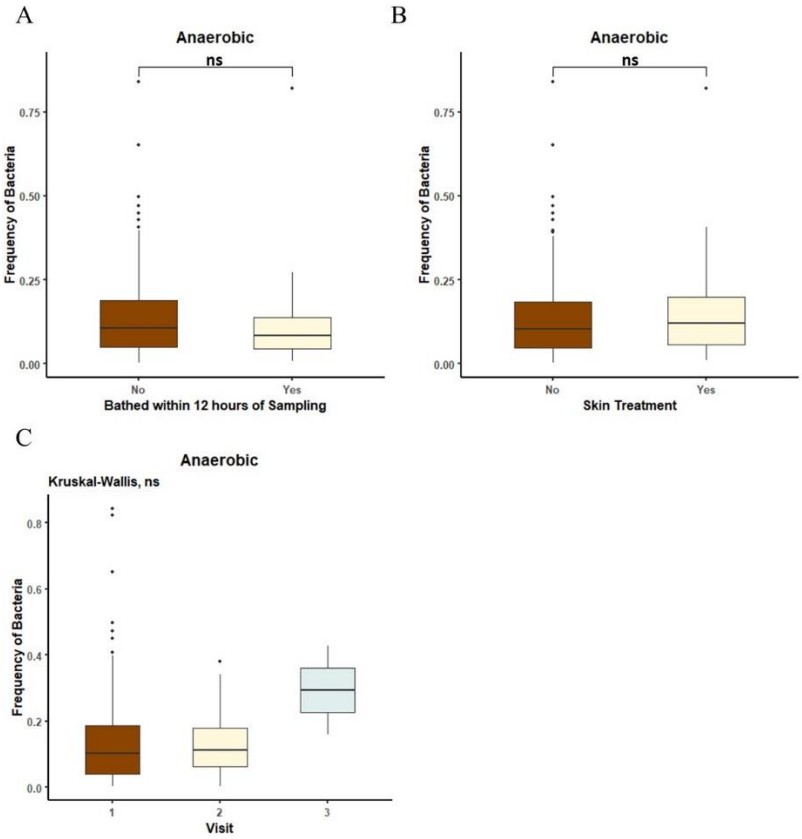
Supplement Figure 7.5 Frequency of each Oxygen Tolerance's Microbiome in ProRaD

Frequency of (A) Facultative anaerobic microbiome and (B) Aerobic microbiome according to health status. Significance determined by Kruskal-Wallis with Dunn's Test as post hoc analysis. Abbreviations and colors: healthy (HE, purple), atopic eczema lesional (AE LS, yellow), and atopic eczema non-lesional (AE NL, burnt yellow) skin.



Supplement Figure 7.6 Frequency of Anaerobic OTU's in ProRaD Relative to Potential Intrinsic Confounding Factors

Dunn's test for multiple comparisons, Mann-Whitney for two group comparisons. (A) Correlation of age to anaerobic microbiome, stratified by health status. Color scheme: HE (purple), AE NL (yellow), AE LS (burnt yellow). (B) Influence of sex on the anaerobic microbiome; female (171, red), male (105, light yellow). (C) Influence of skin microenvironment; dry (68, red), sebaceous (20, light yellow), moist (153, light blue), missing location information (20, white), undetermined microenvironment based on location information (15, teal).



Supplement Figure 7.7 Frequency of Anaerobic OTU's in Relation to Potential Extrinsic Confounding Factors

Dunn's test for multiple comparisons, Mann-Whitney for two group comparisons. (A) Influence of bathing within 12 hours of sampling; no (n = 246, red), yes (30, light yellow). (B) Influence of skin treatment (an emollient) applied within 12 hours of sampling; no (253, red), yes (23, light yellow). (C) Influence of visit; first (225, red), second (49, light yellow), third (2, light blue).

A **Non-lesional**

Factor	Estimate (p-value)
Health Status	-0.05365 (0.04198)
Microenvironment	
Moist	-0.06699 (0.00461)
Sebaceous	-0.01808 (0.62399)
Undetermined	-0.02860 (0.46679)
Treatment	0.09100 (0.02799)
Combined Model	0.08247 R^2 (0.01464)

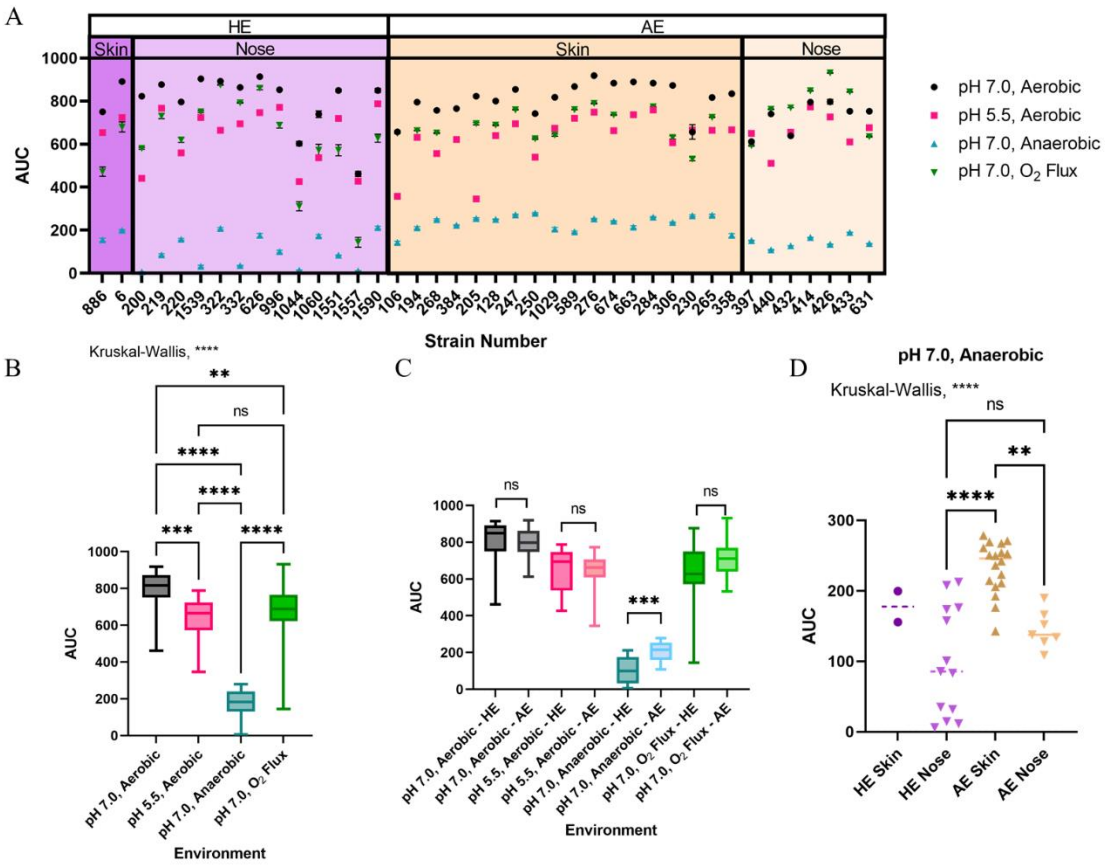
B **Lesional**

Factor	Estimate (p-value)
Health Status	-0.05039 (0.026)

Supplement Figure 7.8 Multiple Regression for Determination of Confounding Factors in Co-occurrence

The best fitted model, determined by backwards and forwards regression is listed here for (A) non-lesional (B) lesional atopic eczema. Data without microenvironment information was excluded.

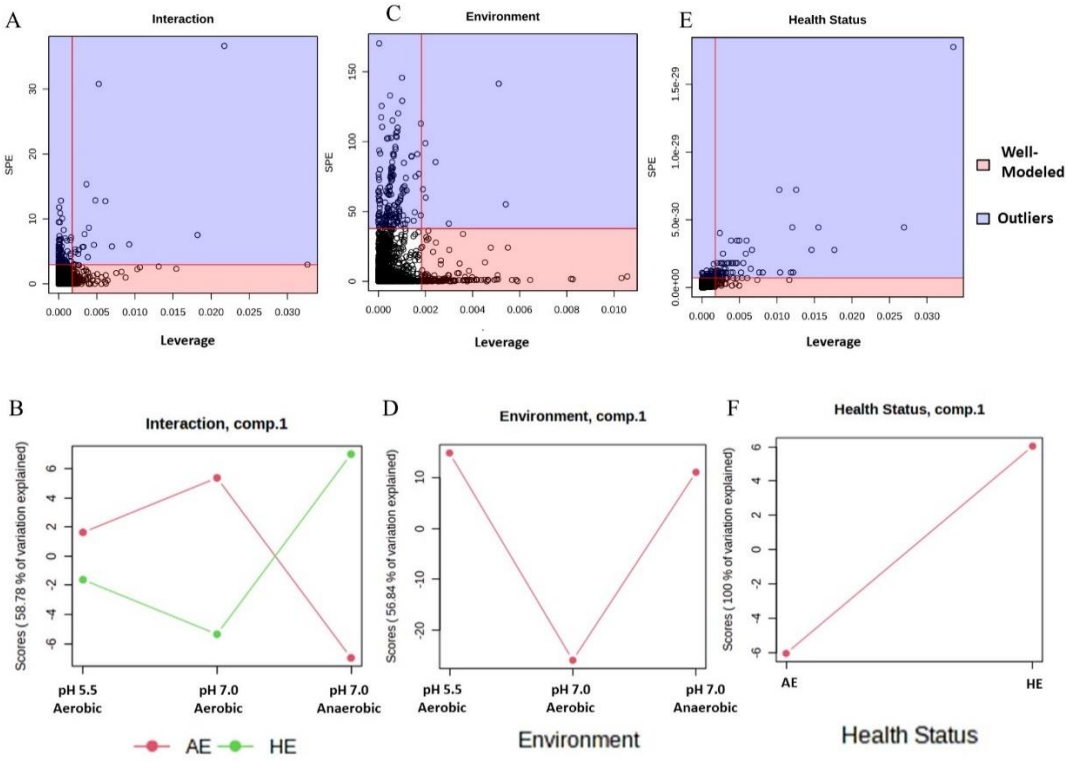
7.1.5 Section 3.3 Supplement



Supplement Figure 7.9 AUC for Growth Curves of *Staphylococcus aureus* Strains

All strains were grown in pH 7.0, Aerobic (black); pH 5.5, Aerobic (pink); pH 7.0, Anaerobic (blue); or pH 7.0, Oxygen Flux (green) conditions. (A) Area under the curve (AUC) of each strain. The purple background indicates healthy (HE) strains and the yellow background indicates atopic eczema (AE) strains. The darker background shades are skin isolates and the lighter shades are nose isolates. (B) Boxplot of AUC in each environment, with post-hoc Dunn's test (C) Boxplot of AUC stratified by HE (darker shades) and AE strains (lighter shades); Mann-Whitney test, not corrected for multiple testing (D) Scatter plot of HE and AE strains according to the location of isolation – skin (darker shades) and nose (lighter shades) grown in pH 7.0, Anaerobic conditions. A line denotes the median, and the significance is calculated by post-hoc Dunn's test.

7.1.6 Section 3.4.2 Supplement



Supplement Figure 7.10 ASCA Analysis

The (A, B) interaction between environment and health status, (C, D) environment, and (E, F) health status alone was modeled according to ASCA. (A, C, E) Squared Prediction Error (SPE)/Leverage plots where the metabolites' contribution to the model (leverage) and how well the metabolite fits into the model (SPE). (B, D, F) The score plots are based on principle component one where the behavior of the groups within each factor is described. To ensure that the model is accurate, permutation tests were performed where each model had the following p-values: interaction ($p = 0.25$), environment ($p < 0.05$), and health status ($p = 0.05$).

Supplement Table 7.2 Significantly Different Metabolites within pH 5.5, Aerobic Between HE and AE Strains

Significantly different metabolites calculated by volcano plot of the pH 5.5, Aerobic subgroup of the full data set. Significantly different was determined if the metabolite had both $p \leq 0.05$ and a fold change higher than 5-fold. Metabolites, also called cluster, were putatively annotated by MassTRIX. All possible annotations are listed, and clusters with no annotation were left blank. The putative annotation was then compared to the data published in Afghani et al. 2021 to determine if it was found in skin.

Cluster ID	Putative Compound ID	Found in Skin
Cluster_0366		-

	2'-Deoxycytidine 4-amino-1-[4-hydroxy-5-(hydroxymethyl)oxolan-2-yl]-pyrimidin-2-one[primary alcohol] N-Acetoxy-4-aminobiphenyl N-Hydroxy-4 acetylaminobiphenyl	No
Cluster_0451	Flindersine	
Cluster_1094		-
Cluster_1298		-
Cluster_1489		-
Cluster_1766	Acetylblasticidin S	No
Cluster_1814		-
Cluster_1857	PD 123177	No
Cluster_2012		-
Cluster_2248	Adonitoxin Aspecioside Convallatoxin	No
Cluster_2498		-
Cluster_3006		-
Cluster_3711		-
Cluster_4354		-
Cluster_4522		-

Supplement Table 7.3 Significantly Different Metabolites within pH 7.0, Aerobic between HE and AE Strains

Chapter 7 -Supplementary Figures

Significantly different metabolites calculated by volcano plot of the pH 7.0, Aerobic subgroup of the full data set. Significantly different was determined if the metabolite had both $p \leq 0.05$ and a fold change higher than 5-fold. Metabolites, also called cluster, were putatively annotated by MassTRIX. All possible annotations are listed, and clusters with no annotation were left blank. The putative annotation was then compared to the data published in Afghani et al. 2021 to determine if it was found in skin.

Cluster ID	Putative Compound ID	Found in Skin
Cluster_0008		-
	Acetate Glycolaldehyde R replaced by H in Aldose Acetyl ester R replaced by H in Fatty acid methyl ester R replaced by H in 2-Hydroxycarbonyl compound ([M-H]-) R replaced by H in Monoterpenol acetate ester	Only derivatives found: Aminoacetic acid Dimethylsulfonioacetate Imidazole-4-acetate Benzoyl acetate 4,4-Bis(4-hydroxyphenyl)-3-hexanone diacetate
Cluster_0115	R replaced by H in Hydroxyaldehyde	
Cluster_0586	Shikimate 3-phosphate Shikimate 5-phosphate	No
Cluster_0641		-
Cluster_0978	Thymidine 5'-phosphate	No
Cluster_0983		-
Cluster_1037		-
Cluster_1064	Chlorpromazine N-oxide Penicillin G	No
Cluster_1094		-
Cluster_1144		-
Cluster_1199		-
Cluster_1235		-

Cluster_1298		-
Cluster_1489		-
Cluster_1572		-
Cluster_1605		-
Cluster_1675		-
Cluster_1684		-
Cluster_1698	Chamuvaritin	No
Cluster_1757		-
Cluster_1766	Acetylblasticidin S	No
Cluster_1775		-
Cluster_1845		-
Cluster_1857	PD 123177	No
Cluster_1924		-
Cluster_2012		-
Cluster_2239		-
Cluster_2248	Adonitoxin Aspecioside Convallatoxin	No
Cluster_2498		-
Cluster_2658		-
Cluster_2950	N-Acetyl-leu-leu-leu-leu-tyr-amide	No
Cluster_3027		-
Cluster_3049	PA(14:1(9Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)) PA(18:3(6Z,9Z,12Z)/18:4(6Z,9Z,12Z,15Z PA(18:3(9Z,12Z,15Z)/18:4(6Z,9Z,12Z,15Z	No

	PA(18:4(6Z,9Z,12Z,15Z)/18:3(6Z,9Z,12Z)) PA(18:4(6Z,9Z,12Z,15Z)/18:3(9Z,12Z,15Z)) PA(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/14:1(9Z))	
Cluster_3522		-
Cluster_4207		-
Cluster_4524		-
Cluster_4611		-
Cluster_4705		-
Cluster_4706		-

Supplement Table 7.4 Significantly Different Metabolites within pH 7.0, Anaerobic between HE and AE Strains

Significantly different metabolites calculated by volcano plot of the pH 7.0, Anaerobic subgroup of the full data set. Significantly different was determined if the metabolite had both $p \leq 0.05$ and a fold change higher than 5-fold. Metabolites, also called cluster, were putatively annotated by MassTRIX. All possible annotations are listed, and clusters with no annotation were left blank. The putative annotation was then compared to the data published in Afghani et al. 2021 to determine if it was found in skin.

Cluster ID	Putative Compound ID	Found in Skin
Cluster_0297	Dibenzo-p-dioxin 2-Hydroxydibenzofuran Dibenzofuran-2-ol	No
Cluster_0497	R replaced by H in N2-Acylated Arg-CH2Cl 1'-Acetoxychavicol acetate	No
Cluster_1385		-
Cluster_1457		-
Cluster_1910		-
Cluster_2018		-

Cluster_2135		-
Cluster_2453	Pubescenol Bilirubin (3Z)-Phytochromobilin 15,16-Dihydrobiliverdin 4E,15Z-Bilirubin IXa	No
Cluster_2461		-
Cluster_3061		-
Cluster_3080		-
Cluster_3655		-
Cluster_3862		-
Cluster_3895	PS(18:2(9Z,12Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z PS(20:4(5Z,8Z,11Z,14Z)/20:4(5Z,8Z,11Z,14Z)) PS(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/18:2(9Z,12Z)) PS(18:4(6Z,9Z,12Z,15Z)/22:4(7Z,10Z,13Z,16Z)) PS(20:3(8Z,11Z,14Z)/20:5(5Z,8Z,11Z,14Z,17Z)) PS(20:5(5Z,8Z,11Z,14Z,17Z)/20:3(8Z,11Z,14Z)) PS(22:4(7Z,10Z,13Z,16Z)/18:4(6Z,9Z,12Z,15Z))	No
Cluster_3973	PE(20:5(5Z,8Z,11Z,14Z,17Z)/24:1(15Z)) PE(22:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z)) PE(22:1(13Z)/22:5(4Z,7Z,10Z,13Z,16Z)) PE(22:1(13Z)/22:5(7Z,10Z,13Z,16Z,19Z)) PE(22:2(13Z,16Z)/22:4(7Z,10Z,13Z,16Z)) PE(22:4(7Z,10Z,13Z,16Z)/22:2(13Z,16Z)) PE(22:5(4Z,7Z,10Z,13Z,16Z)/22:1(13Z)) PE(22:5(7Z,10Z,13Z,16Z,19Z)/22:1(13Z)) PE(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:0) PE(24:1(15Z)/20:5(5Z,8Z,11Z,14Z,17Z))	No

PC(19:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z)) PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/19:0)	
Cluster_4173	-
Cluster_4302	-
Cluster_4354	-
Cluster_4650	-

7.2 List of Talks and Posters

7.2.1 Oral Presentations

Supplement Table 7.5 List of Oral Presentations

Title	Conference	Year
Environmental impact on *S. aureus*: Secretome differences between Healthy and Atopic Eczema Isolates	49th Arbeitsgemeinschaft Dermatologische Forschung	2023
New Developments in Eczema Research: Skin Metabolome Sampling and Skin Bacterial Secretions; Oxygen Exposure and pH Stability	European Academy of Allergy and Clinical Immunology Summer Symposium on Epithelial Barriers and Microbiome	2022
Easy Non-invasive Clinical Sampling for the Diagnosis of Skin Disease	34th Mainzer Allergie Workshop	2022
Breaking Down Barriers: Understanding Anaerobic Bacteria in the Skin	ZIELkolaus – Nutrition and Microbiome	2019

7.2.2 Poster Presentations

Supplement Table 7.6 List of Poster Presentations

Chapter 7 -List of Talks and Posters

Title	Conference	Year
Microbiome and its Metabolome: New methods to See Function in Atopic Eczema	Wissenschaftstag Symposium	2022
Accurate Representation of Bare Skin Metabolome by Non-Invasive WET-PREP Sampling	16th World Immune Regulation Meeting	2022
Influence of Microbial Secretion and Clinical Skin Metabolome Sampling in Atopic Eczema	Helmholtz Microbiome Day 2022	2022
Enhanced Access to the Health-related Skin Metabolome by Fast, Reproducible and Non-invasive WET PREP Sampling	16th Annual Conference – Metabolomics Society	2021
Oxygen as a Factor in Atopic Eczema	47th Arbeitsgemeinschaft Dermatologische Forschung Conference	2021
Enhanced Access to the Health-related Skin Metabolome by Fast, Reproducible and Non-invasive WET PREP Sampling	13th Seeon Conference - Microbiota, Probiotics and Host	2021
Methodological Collection Influence in Skin Metabolome	Wissenschaftstag Symposium	2020
Breaking Down Barriers Understanding Anaerobic Bacteria in the Skin	Augsburger Neurodermitis Symposium	2019

Breaking Down Barriers: Understanding Anaerobic Bacteria in the Skin	12th Seeon Conference - Microbiota, Probiotics and Host	2019

7.3 Publication List

Afghani J, Traidl-Hoffmann C, Schmitt-Kopplin P, Reiger M, Mueller C. An Overview of the Latest Metabolomics Studies on Atopic Eczema with New Directions for Study. Int J Mol Sci. 2022 Aug 8;23(15):8791. doi: 10.3390/ijms23158791. PMID: 35955924; PMCID: PMC9368995

Afghani J, Huelpuesch C, Schmitt-Kopplin P, Traidl-Hoffmann C, Reiger M, Mueller C. Enhanced Access to the Health-Related Skin Metabolome by Fast, Reproducible and Non-Invasive WET PREP Sampling. Metabolites. 2021 Jun 24;11(7):415. doi: 10.3390/metabo11070415. PMID: 34202850; PMCID: PMC8304125.

Williams LM, Inge MM, Mansfield KM, Rasmussen A, **Afghani J**, Agrba M, Albert C, Andersson C, Babaei M, Babaei M, Bagdasaryants A, Bonilla A, Browne A, Carpenter S, Chen T, Christie B, Cyr A, Dam K, Dulock N, Erdene G, Esau L, Esonwune S, Hanchate A, Huang X, Jennings T, Kasabwala A, Kehoe L, Kobayashi R, Lee M, LeVan A, Liu Y, Murphy E, Nambiar A, Olive M, Patel D, Pavesi F, Petty CA, Samofalova Y, Sanchez S, Stejskal C, Tang Y, Yapo A, Cleary JP Jr, Yunes SA, Siggers T, Gilmore TD. Transcription factor NF-κB in a basal metazoan, the sponge, has conserved and unique sequences, activities, and regulation. Dev Comp Immunol. 2020 Mar; 104:103559. doi: 10.1016/j.dci.2019.103559. Epub 2019 Nov 18. PMID: 31751628.