December 2014

The effect of probiotics on skin

Probiotics in dermatology — from theory to enterprise

ACADEMIC CONSULTANCY TRAINING
Group 1457

Bob van den Berg
Jiang Chang
Aafke Duizendstra
Renate Jansen
Ana Jimena Pacheco Gutierrez
Tian Zhao

Coach: Carel Weijers
Content coach: Willemien Lommen
Commissioner: Skinwiser, Dr Jetske Ultee & Matthijs Boog

Ana Jimena Pacheco Gutierrez
ana.pachecogutierrez@wur.nl
0626667543

Stichting Skinwiser
Matthijs Boog (matthijs@dr-jetskeultee.nl)
Maasstraat 11
3016 DB Rotterdam

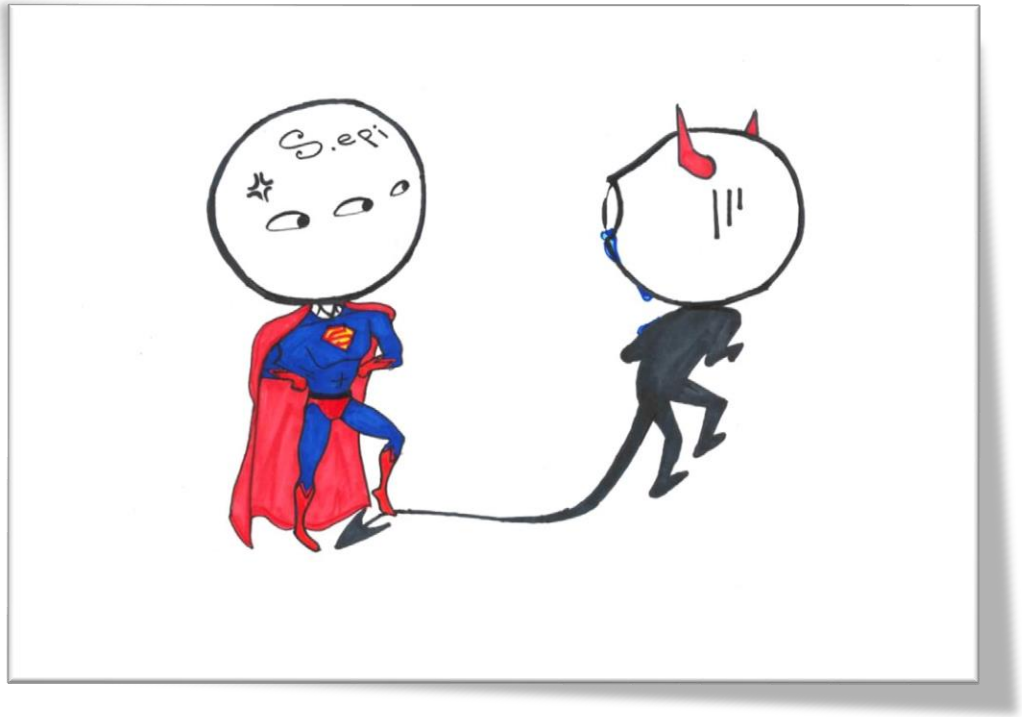
Source image: " The trillions of microbes in and on our bodies are key to understanding our health " Follow Up on AO+ Living Bacterial Skin Tonic - Allergies & Your Gut (Accessed December 5, 2014)

Disclaimer

Part of MSc course Academic Consultancy Training (9 ECTS)

The effect of probiotics on skin

ACT group 1457



Team logo of *ACT group 1457*, all rights reserved

" *Staphylococcus epidermidis* protects our skin from the invading pathogens "

Executive summary

This project was commissioned by Skinwiser to examine whether probiotics can have a positive effect on the skin. We assessed if topical application of probiotics can improve healthy or affected skin. The skin conditions included are rosacea, acne and atopic dermatitis. First, literature about healthy skin, skin conditions and (gut) probiotics was studied. Based on that, we pinpointed mechanisms in healthy and affected skin that could be targeted using probiotics. Next, we looked for bacterial strains that could tackle the selected mechanisms. Simultaneously, we performed a short market study on (non-oral) probiotics.

We conclude that healthy skin could be best targeted by improving skin barrier function using sphingomyelinase-producing *Streptococcus thermophilus*, thereby increasing ceramide levels in the skin. No suitable strains were found for improving rosacea. In acne, *Propionibacterium acnes* overgrowth could be tackled by inhibiting this bacterium using *Staphylococcus epidermidis*.

Atopic dermatitis could be improved using sphingomyelinase-producing *Streptococcus thermophilus*. Furthermore, inhibiting *Staphylococcus aureus* using Esp-secreting *Staphylococcus epidermidis* could improve atopic dermatitis symptoms. Moreover, clinical studies using *Vitreoscilla filiformis* lysate showed promise in improving atopic dermatitis, potentially by improving immune tolerance.

We also conclude that the market study showed that most available products were intended for disease prevention, healthy skin or skin cleaning. Information about specific strains included was rarely disclosed and claimed working mechanisms were mostly competitive exclusion of pathogens and strengthening of the skin.

In our opinion probiotics can have a positive effect on the skin. However, more research is needed to verify the efficacy of the strains found. We recommend further research into the previously mentioned strains for the development of probiotics–containing skin care products. Oral probiotics are more promising to target rosacea than topical probiotics based on current research.

A combination of pre- and probiotics could be used to increase their effect, while a combination of pro- and postbiotics could include products of pathogenic bacteria. Inclusion of spores from spore-forming bacteria can be a promising approach to store microorganisms in a product.

We advice Skinwiser to research (some or all of) the selected strains. Recommendations for further research are included to help in this process.

Abbreviations

AD	:	Atopic dermatitis
AMP	:	Antimicrobial protein
APC	:	Antigen Presenting Cell
APN	:	Amino-peptidase N
CE	:	Cornified envelope
CFMW	:	CM4-fermented milk whey
CGRP	:	Calcitonin gene related peptide
CRH	:	Corticotrophin Releasing Hormone
DCs	:	Dendritic cells
DHEA	:	Dehydroepiandrosterone
DHT	:	Dihydrotestosterone
DP-IV	:	Dipeptidyl peptidase IV
EGFβ	:	Epidermal growth factor beta
Esp	:	Extracellular serine protease
ETR	:	Erythematotelangiectatic Rosacea
FAO	:	Food and Agriculture Organization
FDA	:	Food and Drug Administration
FOS	:	Fructo-oligosaccharides
GI	:	Gastro-intestinal
GOS	:	Galacto-oligosaccharides
GST	:	Glutathione S-transferase
hBD2	:	Human β-defensin-2
IBD	:	Inflammatory bowel disease
IBS	:	Irritable Bowel Syndrome
IFNγ	:	Interferon γ
IL-12	:	Interleukin-12
IMO	:	Isomalto-oligosaccharide
ITF	:	Nulin-type fructants
KLK5	:	Kallikrein 5
LC	:	Langerhans Cells
LGG	:	*Lactobacillus rhamnosus* GG
LRP	:	La Roche Posay
MAMP	:	Microbe Associated Molecular Pattern
MAP	:	Mitogen activated protein
mEASI	:	Modified eczema area severity index
MED	:	Minimal Erythemal Dose
MMPs	:	Matrix metalloproteinases
MRSA	:	Methicillin-resistant *Staphylococcus aureus*
NFκB	:	Nuclear factor κB
NK cells	:	Natural Killer cells
NMF	:	Natural Moisturizing Factor
PAR-2	:	protease-activated receptor-2
PPR	:	Papulopustular Rosacea
PRR	:	Pattern Recognition Receptor
ROS	:	Reactive Oxygen Species
SIBO	:	Bacterial Overgrowth in Small Intestine
SCFAs	:	Short chain fatty acids
SCORAD:		SCORing Atopic Dermatitis
SOD	:	Superoxide dismutases
SP	:	Substance P
SST	:	Somatostatin
TEWL	:	Transepidermal water loss
Th-cells	:	T helper cells
TLR	:	Toll-like Receptor
T-regs	:	Regulatory T-cells
TRPV1	:	Transient receptor potential vanilloid subfamily member 1
UVB	:	Ultraviolet B
VIP	:	Vasoactive Intestinal Peptide
WHO	:	World Health Organization
YopP	:	Yersinia outer protein P

Table of Contents

1. INTRODUCTION 3
1.1 Introduction to the report 3
1.1.1 Scope 3
1.1.2 Organization of the report 4
1.2 Background 5
1.2.1 Overview of the immune system 5
1.2.2 Host-microbe interactions 10
1.2.3 Gut microbiome 11
1.2.4 Probiotics, prebiotics and postbiotics 13
2. METHODOLOGY 20
3. RESULTS 23
3.1 Healthy skin 23
3.1.1 The skin 23
3.1.2 Microbiome on the skin 25
3.1.3 Photoprotection by oral probiotics 26
3.2 Rosacea 30
3.2.1 Symptoms 30
3.2.2 Quality of life patients 31
3.2.3 Risk factors 32
3.2.4 Molecular mechanisms 36
3.2.5 Interactions between the possible risk factors and molecular mechanisms 39
3.2.6. Treatments 40
3.2.7 Summary rosacea 44
3.3 Acne 45
3.3.1 Symptoms 45
3.3.2 Quality of life patients 46
3.3.3 Causes 46
3.3.4 Treatments 50
3.3.5 Summary acne 51
3.4 Atopic dermatitis 52
3.4.1 Symptoms 52
3.4.2 Treatments 53
3.4.3 Genetics and environment 53
3.4.4 Important factors in atopic dermatitis 54
3.4.5 Summary atopic dermatitis 60

3.5 Market study 61
3.5.1 Skin treatment 62
3.5.2 Cleaning products 63
3.5.3 Allergen removing spray 64
4. DISCUSSION 65
4.1 Targeting healthy skin 65
4.1.1 Bacterial production of beneficial compounds 65
4.1.2 Improving skin barrier function 66
4.1.3 Summary and recommendation healthy skin 66
4.2 Targeting rosacea 66
4.2.1 Overexpression of NFκB 67
4.2.2 Increased ROS levels 67
4.2.3 Increased neutrophil activity 67
4.2.4 Increased activity of Staphylococcus epidermidis and TLR2 on the skin 68
4.2.5 Increased pH 68
4.2.6 Summary and recommendation rosacea 69
4.3 Targeting acne vulgaris 69
4.3.1 Inflammation 69
4.3.2 Propionibacterium acnes overgrowth 69
4.3.3 Summary and recommendation acne 70
4.4 Targeting atopic dermatitis 70
4.4.1 Staphylococcus aureus infection 70
4.4.2 Decreased ceramide levels 71
4.4.3 Decreased immune tolerance 72
4.4.4 Summary and recommendation AD 73
4.5 Market study 73
4.6 Overall discussion 74
5. CONCLUSIONS AND ADVICE 75
6. RECOMMENDATION FOR FURTHER RESEARCH 78
ACKNOWLEDGEMENTS 80
REFERENCES 81
APPENDIX I

1. INTRODUCTION

1.1 Introduction to the report

This report presents the outcomes of the consultancy project 'Probiotics in dermatology – from theory to enterprise'. This project was commissioned by the Skinwiser foundation to examine whether probiotics can have a positive effect on the skin. We assessed if topical application of probiotics can improve healthy or affected skin. The skin conditions included are rosacea, acne and atopic dermatitis.

1.1.1 Scope

Problem description

The development of new skin care products containing only ingredients that are scientifically proven to be effective is one of the main goals of the Skinwiser foundation and associated organizations (Uncover Skincare and Sunwiser). As defined by the World Health Organization, probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). Research has shown that probiotics have beneficial effects on certain intestinal diseases by influencing the gut microbiome (see section *1.2.4 Probiotics, prebiotics and postbiotics*). The skin has its own microbiome, hence the use of probiotics in dermatology also seems promising. **Skinwiser is interested in *knowing whether probiotics can have a positive effect on the skin*.** The foundation is especially interested in the effects of topically applied probiotics on commonly occurring skin conditions such as rosacea, acne and atopic dermatitis, as well as their general preventative effects on healthy skin.

An imbalance in the skin microbiome, called dysbiosis, has been observed in various conditions, including atopic dermatitis (Sanford and Gallo, 2013). Although it is not entirely clear in these cases whether the pathology caused the dysbiosis, or dysbiosis the pathology (Sanford and Gallo, 2013), resolving or improving dysbiosis in skin conditions could improve symptoms. Similarly, maintaining a balanced skin microbiome using probiotics could make the microbiome more resilient to for instance infections or improve skin barrier function.

Currently, antibiotic drugs are frequently used to treat acne, rosacea and atopic dermatitis. Antibiotics likely have a large influence on the microbes living on our skin, as they are often nonspecific and kill not only pathogenic microorganisms, but also non-pathogenic ones, thereby greatly disrupting the skin microbiome (Reid *et al.*, 2011). Probiotics could be a milder, less aggressive alternative to antibiotics for restoring homeostasis of the skin microbiome (Reid *et al.*, 2011).

Probiotics can either be applied topically or ingested orally. Skinwiser intends to develop their own probiotics-containing skin care products, as part of Uncover Skincare. Therefore, our focus is on topical application of probiotics and probiotic products.

Purpose

The purpose of this project is to inform and advise the Skinwiser foundation on the use of probiotics in skin care, as well as on if and how to proceed with further research. Research questions that will be answered by a first literature review include:

- Which mechanisms are responsible for the effects of probiotics in the gut?
- What is the core microbial composition of healthy human skin?
- What is the pathophysiology of rosacea?
- What is the pathophysiology of acne?
- What is the pathophysiology of atopic dermatitis?

Questions that will be answered by a short market study are:

- Which products containing probiotics are currently available on the international market?
- What are the (active) probiotic ingredients in these products, if known?
- How effective are these probiotic ingredients, if known?

Based on the answers to the previous questions, we will identify mechanisms important in the maintenance of healthy skin and in the pathophysiology of the skin conditions. For the selected mechanisms, we will carry out a second literature search on microorganisms influencing these mechanisms. The outcome of this search is the basis for our advice on whether or not probiotics could be used in dermatology.

Background and philosophy Skinwiser foundation and associated organizations

The Skinwiser foundation is a skin research institute established by Dr. Jetske Ultee, who is a research physician in cosmetic dermatology and is especially interested in the skin. Research topics of Skinwiser include the stability of antioxidants in cosmetics and the effectivity of oral and topical use of botanicals in protection against the sun. Via her blog, www.jetskeultee.nl in Dutch and www.jetskeultee.com in English, Jetske Ultee wants to share her knowledge about skin care with the public. Furthermore, she founded the Sunwiser foundation, a non-profit organization that researches sunbathing habits and aims to educate people about safety in the sun. Sunwiser has also developed a safe and effective sunscreen. This sunscreen is part of a line of products produced by Uncover Skincare developed by Jetske Ultee. Besides this sunscreen, Uncover Skincare also sells other skin care products, e.g. moisturizers, cleansers and toners. The products of Uncover Skincare contain only ingredients that have been proven to be effective, at optimal concentrations and are free from irritants.

Goals

The goal of this project is to improve commonly occurring skin conditions and healthy skin. The aim of this project is to advise Skinwiser on whether or not probiotics can be used to improve symptoms of common skin conditions and prevent complaints in healthy skin. This will be based on current scientific knowledge. In addition, we describe which probiotics are currently the most promising for treating skin diseases and healthy skin.

1.1.2 Organization of the report

The organization of the report is as follows:

- Background
- Methodology
- Results
    - Healthy skin and skin microbiome
    - Pathophysiology of rosacea, acne and atopic dermatitis

    - Market study
- Discussion
    - Target mechanisms
    - Market study
- Conclusions and advice
- Reccomendations for further research

1.2 Background

1.2.1 Overview of the immune system

The immune system plays a central role in the effect of probiotics on human health. Therefore we include a brief overview of the main aspects of the immune system here, to refer back to when reading this report.

The immune system eradicates pathogens and tries to prevent disease. It consists of the innate immune system and the adaptive immune system. The innate immune system detects infection and determines the nature of infection; it is the first line of defence and is the initiator of the adaptive immune response (Medzhitov, 2001). The adaptive immune system recognizes specific protein sequences and has a specific response.

The innate immune system

The innate immune system consists of several immune cells (see *Figure 1*). These immune cells recognize microbe associated molecular patterns (MAMPs) that are present on almost every bacterium, virus or yeast. MAMPs are essential for the survival of microbes and therefore cannot afford to mutate. In this way, MAMPs are conserved among microbes. These MAMPs are recognized by pattern recognition receptors (PRRs) which are present on cells and inside of cells. These receptors cannot distinguish between pathogenic and commensal microbes.

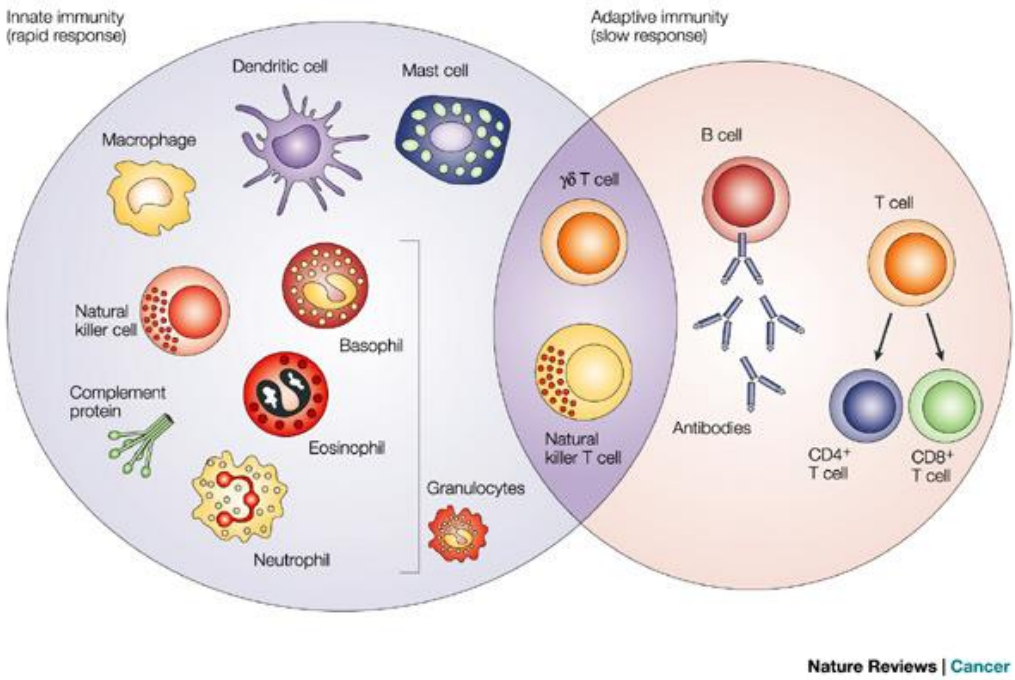


Figure 1. The immune cells in innate and adaptive immune system. Source: Dranoff, 2004.

There are extracellular and intracellular PRRs e.g. Toll-like receptors (TLRs) (Medzhitov, 2001), Nod-like receptors (NLRs) and Rig-like receptors (RLRs) (Abbas *et al.,* 2012). In *Figure 2* an overview of the mammalian TLRs and their ligands is given. TLR3, TLR7, TLR8 and TLR9 are intracellular receptors which recognize viruses and intracellular bacteria and parasites. TLR1, TLR2, TLR4, TLR5, TLR6 and TLR11 are extracellular receptors and recognize extracellular bacteria and parasites. When a TLR receptor is bound, a signalling cascade results in the induction of inflammatory cytokines (e.g. interleukins) and chemokines, antimicrobial peptides and other molecules that act against the invading pathogens. The TLRs activate distinct but also overlapping cellular pathways (Janeway and Medzhitov, 2002).

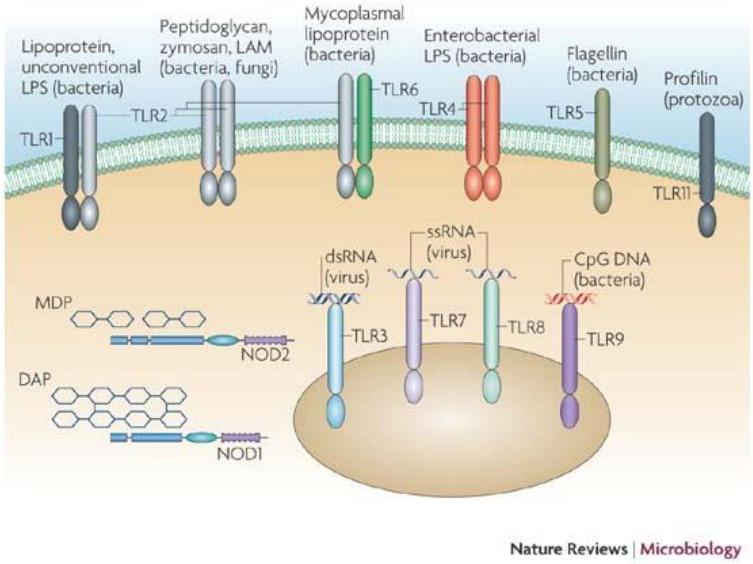


Figure 2. TLRs and their ligands. Source: Kaufmann, 2007.

The skin is lined by a continuous layer of epithelia and forms a barrier against microbes that try to enter the body (Abbas *et al.,* 2012). When microbes enter, they encounter cells of the innate immune

system. A process called inflammation occurs: the recruitment of leukocytes and plasma proteins from the blood into the tissue. Leukocytes are white blood cells e.g. neutrophils and macrophages. Many cytokines and chemokines are involved in inflammation. Immune cells are made of two different lineages: the lymphoid lineage and the myeloid lineage (see *Figure 3*). The innate immune cells (see *Figure 1*) are derived from the myeloid lineage and consist of e.g. monocytes (macrophages), neutrophils, eosinophils, dendritic cells.

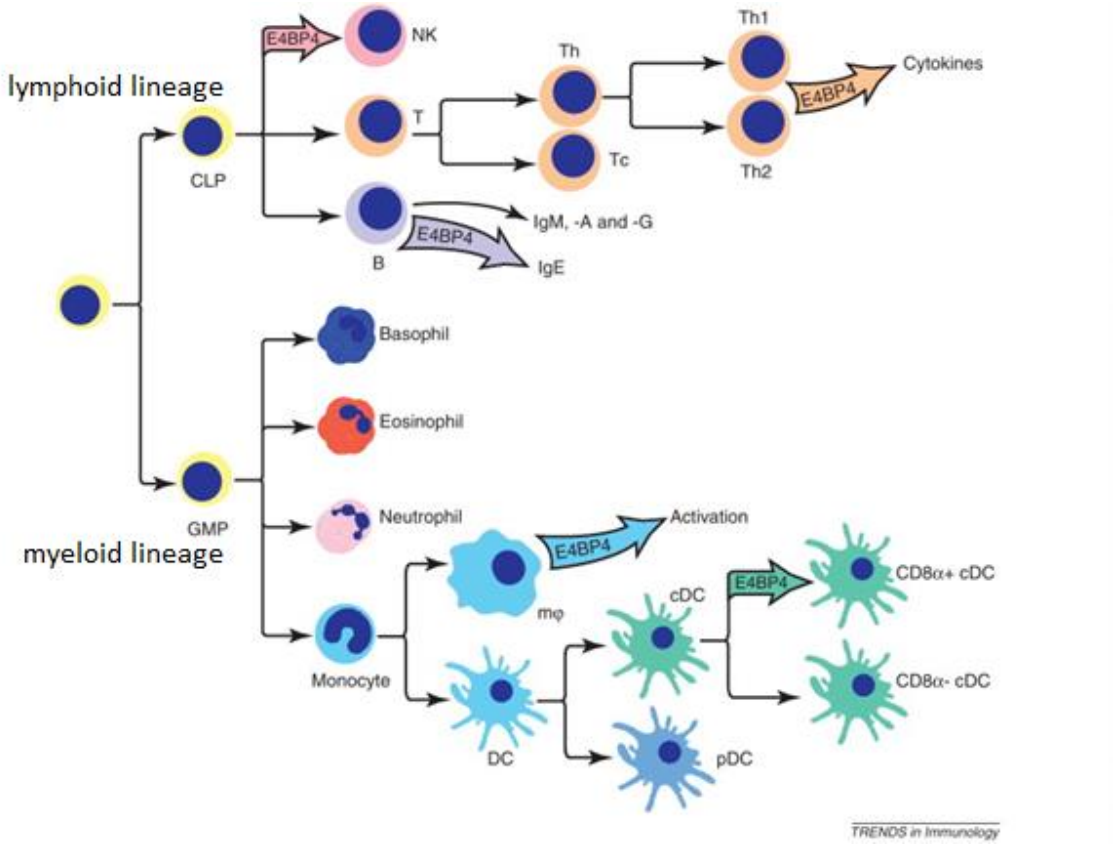


Figure 3. The cells of the immune system and their lineage. NK: natural killer cell; T: T-cell; B: B-cell; Th: T-helper cell; IgE, IgM, IgA, IgG: antibodies; mφ: macrophage; DC: dendritic cell. Source: modified from Male *et al.*, 2012.

Monocytes travel in the blood until they interact with specific chemokines that attracts them to the tissue (Abbas *et al.,* 2012). The monocytes then migrate into the tissue and become macrophages. Macrophages engulf microbes and kill them using ROS (reactive oxygen species) and nitric oxide. They also secrete cytokines that instruct other cells what to do. Furthermore, they are antigen presenting cells (APCs) that present antigens to T-lymphocytes thereby activating these T-cells. Antigens are specific parts of the microbe, usually a protein sequence, against which an adaptive immune response can be formed (Abbas *et al.,* 2012).

Neutrophils are the most abundant white blood cells in the body and are present in early inflammation (Abbas *et al.,* 2012). They contain two types of granules. These granules contain substances that kill microorganisms. One type of granule contains enzymes like elastase, collagenase and lysozyme, the other type contains cathelicidins and defensins. The neutrophils only circulate in the blood for 6 hours. If they do not migrate to a site of infection during that time, they go into apoptosis (programmed cell death) (Abbas *et al.,* 2012).

Mast cells are present in the skin and contain granules filled with histamine and other inflammatory and antimicrobial factors (Abbas *et al.,* 2012). They have IgE and IgG receptors on their cell surface. When these receptors are bound by e.g. allergens, the granules release their content into the extracellular space. This process causes inflammation (Abbas *et al.,*2012).

Eosinophils have granules that contain enzymes harmful for both host tissue and cell walls of parasites and bacteria. Some eosinophils are present in mucosal linings of the body, but the amount can increase when there is inflammation (Abbas *et al.,* 2012).

Dendritic cells (DCs) are the most important immune cells because they connect the innate and adaptive immune system. When activated by microbes, they are APCs and activate T-cells (Abbas *et al.,* 2012).

The adaptive immune system

The adaptive immune system can distinguish between different microbial molecules (antigens). It has a memory and therefore responds faster and stronger upon repeated exposure to the same antigen (Abbas *et al.,* 2012). The adaptive immune system comprises different cells (see *Figure 1*) that are derived from the lymphoid lineage (see *Figure 3*).

CD4+ helper T-cells (Th-cells) are one subset of T-cellzs (Abbas *et al.,* 2012). DCs present antigens to naive T-cells and this results in the development of effector T-cells like Th-cells. A DC will direct T-cell differentiation into different types of Th-cells(e.g. Th1, Th2) depending on the type of microbe it encountered. *Figure 4* depicts how different cytokines induce naive T-cells to develop into different types of Th-cells, the cytokines that the Th-cells secrete and how in turn these cytokines influence T-cell differentiation.

When DCs secrete interleukin-12 (IL-12) and interferon γ (IFNγ), naive T-cells differentiate into Th1 cells. IFNγ induces DCs to produce more IL-12. Furthermore, Th1 cells produce IFNγ and thus amplify this reaction. In addition, IFNγ inhibits the differentiation of naive cells into Th2 and Th17 cells (see *Figure 4*). IFNγ produced by the Th1 cells activate macrophages (Abbas *et al.,* 2012).

When IL-4 is secreted by mast cells and/or other cell populations, Th2 cells develop from naive T-cells (see *Figure 4*). These T-cells are produced in response to allergens and helminths. Th2 cells produce IL-4 themselves, so this reaction also amplifies itself. Furthermore, IL-4 inhibits the differentiation of T-cells into Th1 cells and Th17 cells. Th2 cells secrete IL-4, which stimulates IgE production by B-cells, IL-5, which stimulates eosinophils, and IL-13. Mast cells are activated by IgE coated antibodies. IL-4 and IL-13 activate alternative macrophages that contribute to tissue remodelling and fibrosis (Abbas *et al.,* 2012).

Naive CD4+ T-cells can also differentiate into regulatory T-cells (T-regs, see *Figure 4*). T-regs are formed by the presence of IL-2 and transforming growth factor β (TGFβ). They can bind to APCs and thereby inhibit the APC stimulation of T-cells. T-regs produce TGFβ and IL-10, which inhibit the immune response. TGFβ inhibits the activation of macrophages, Th1 and Th2 cells and, in combination with IL-1 and IL-6, promotes the development of Th17 cells. IL-10 inhibits T-cell activation and the production of IL-12 by macrophages and DCs (Abbas *et al.,* 2012). In conclusion, T-regs inhibit the immune response by inhibiting Th1 cells, Th2 cells, macrophages and DCs. When there is a lack of T-regs, systemic inflammation or autoimmune diseases can occur (Abbas *et al.,*

2012). Some T-cells also develop into memory T-cells so the reaction in the next encounter with the same antigen will be faster and better. In normal human skin there is an abundant population of T-cells in the dermis, 95% of which are memory T-cells.

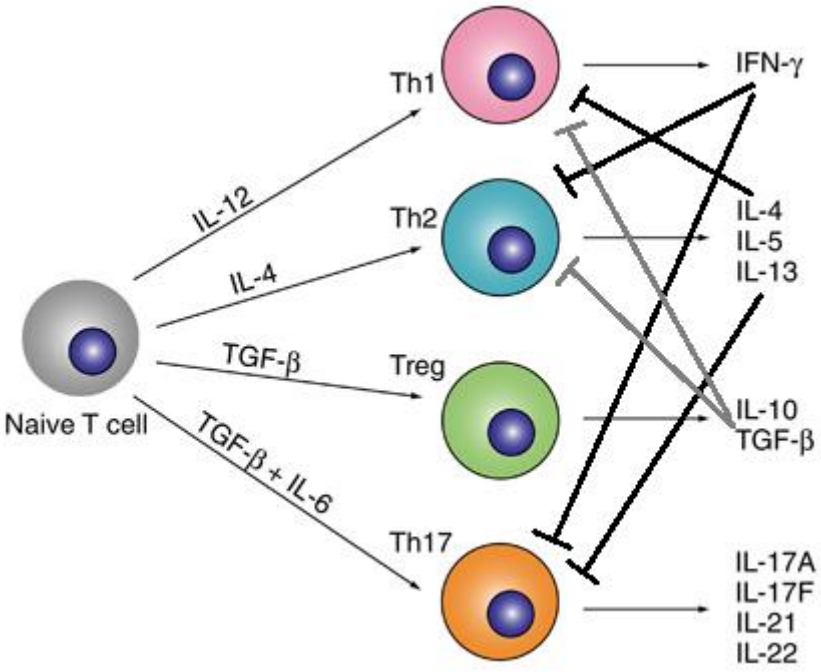


Figure 4. Naive T-cells differentiate into Th-cells according to the type of cytokine Source: modified from Maniati *et al.*, 2010. Different interleukins (IL) and transforming growth factor β (TGFβ) induce several differentiations of T-cells. Th1 cells produce interferon γ (IFNγ), Th2 cells produce IL-4, IL-5 and Il-13 and T-regs produce IL-10 and TGFβ. IFNγ inhibits the differentiation of T-cells into Th2 and Th17 cells while IL-4 inhibits the differentiation of T-cells into Th1 and Th17 cells. TGFβ inhibits the differentiation of T-cells into Th1 and Th2 cells and therefore maintains a balance in the immune response.

B-cells turn into plasma cells or memory B cells when antigens bind to the B-cell receptors. B-cells are stimulated by receptors of Th-cells and cytokines produced by Th-cells. Plasma cells (called after the bloodplasma in which they reside) produce antibodies: this is called the humoral immune response. The type of antibody e.g. IgE, IgM and IgG is dependent on the type of microbe encountered and thus the type of Th cell developed. It also depends on whether it has been encountered before (memory or not) and the anatomical location where it is encountered (e.g. in the gut IgA is produced and in the blood IgM and IgG are produced) (Abbas *et al.,* 2012).

Skin immune system

The skin epidermis (outer layer) forms a physical barrier to microbes and is the site of many immune responses. Keratinocytes are skin cells that respond to pathogens (which are bound on their TLRs) and injury by producing antimicrobial peptides like cathelicidins and cytokines like IL-6, IL-18, IL-1, IL-10, GM-CSF (granulocyte-macrophage colony-stimulating factor) and TNFα (tumour necrosis factor α) (Abbas *et al.,* 2012). GM-CSF activates and differentiates DCs into skin-specific DCs. IL-6, IL-18, IL-1 and TNFα promote inflammation and IL-10 controls immune responses. Furthermore, keratinocytes can produce chemokines that recruit lymphocytes (Abbas *et al.,* 2012).

Langerhans cells (LC) are a type of dendritic cells present in the skin. They form a regular and almost closed network in the epidermis. There are more than 10^9 cells present in the skin and they act like APCs (Bos and Kapsenberg, 1986). In normal intact skin, LCs support proliferation and activation of

resident T-regs. This provides a brake on an inappropriate immune response of foreign antigens and auto-antigens. When the skin barrier is disrupted and pathogens enter the body, the innate immune system provides danger signals (cytokines). With these cytokines, LCs are capable of presenting antigens to T-cells and activate them (Seneschal *et al.,* 2012).

1.2.2 Host-microbe interactions

Interactions between organisms

Interactions between different species can have a positive or negative effect or have no impact on an involved organism. Different combinations of these outcomes can be classified into different interaction types (see *Figure 5*). A general term for these types of interactions is symbiosis, although this term is also often used to describe a situation in which both species benefit. However, this is formally called mutualism. The opposite of mutualism is competition: one species competes with the other species for resources. If one species benefits, but the other is unaffected, this is called commensalism. Parasitism occurs when one species benefits while the other is harmed. Similar is predation, in which one species preys on another species. Amenalism occurs when one species is harmed without any advantage to the other (Cogen *et al.*, 2008; Faust and Raes, 2012). Finally, when there is no net effect on either partner this is called neutralism (Bronstein, 1994).

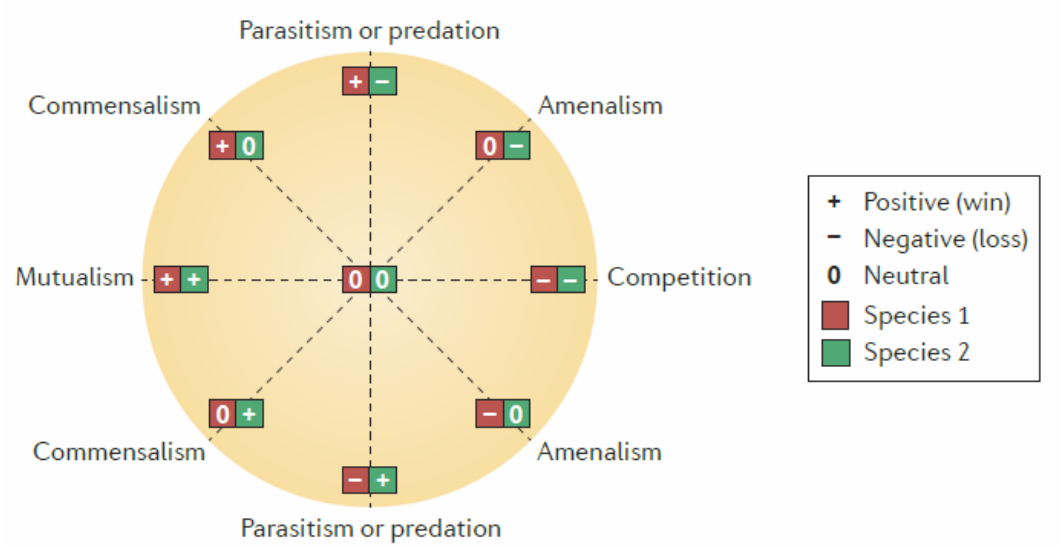


Figure 5. Summary of interactions between members of different species. The effect for each interaction partner can be either positive (+), negative (-) or neutral (0). Terms are further explained in the text. Source: Faust and Raes, 2012.

In real life however, it is not always as clear-cut as this. Most microorganisms colonizing the skin are harmless or even beneficial (Grice and Segre, 2011). Moreover, the same microbe may take on different roles at different times (Cogen *et al.*, 2008). Often the distinction between what is considered a harmless microorganism and pathogenic one depends on the skin's capacity to resist infection and not necessarily on the inherent properties of the microbe (Cogen *et al.*, 2008). Many different complementary systems determine the host cutaneous defence. This includes the physical skin barrier, a hostile surface pH and the production of host defence molecules like antimicrobial peptides, proteases, lysozymes, cytokines and chemokines. Cytokines and chemokines play an important role in activating the cellular and adaptive immune responses (Cogen *et al.*, 2008).

It is very likely that microbes and their hosts, in this case us, have co-evolved. Microbes profit from their hosts through nutrients and a stable ecological niche (Schommer and Gallo, 2013), whereas the host can profit for instance from the capacity of gut microbes to break down (for the host) indigestible food components, thereby increasing the energy uptake (Lin et al., 2014).

Resident versus transient microbes on the skin

The skin microbiome can be divided into two groups: resident and transient microbes. Resident microbes are a relatively fixed group that are routinely found on the skin and that re-establish themselves after perturbations. Often these microorganisms are considered commensal. Transient microbes arise from the environment and persist for hours to days, but do not establish themselves permanently on the surface (Kong and Segre, 2012). The dominant types of bacteria residing at specific sites on the skin of an individual seem to be quite stable over time, while the rare and less abundant types are more transient and differ from day to day (Grice *et al.*, 2009; Grice, 2014).

Under normal circumstances, neither resident nor transient microbes are pathogenic. These normal circumstances are proper hygiene, normal resident flora, controlled immune response and an intact skin barrier function. However, resident and/or transient microbes can colonize, proliferate and cause diseases if perturbations occur (Kong and Segre, 2012). For example, the skin commensal *Staphylococcus epidermidis* can act as an opportunistic pathogen (Otto, 2009).

1.2.3 Gut microbiome

The main focus of this report is on probiotics applied to the skin. However, most research concerning probiotics has been on the gut microbiome. Knowledge of the gut microbiome could provide us with important clues to the effects and interactions of the skin microbiome. Therefore, we include a summary of current knowledge about the gut microbiome, interactions between the gut microbiome and host and mechanisms of restoration of the gut microbiome.

Human gut microbiome

The human gastrointestinal tract is inhabited by nearly 100 trillion (10^{14}) microorganisms (Tojo *et al.*, 2014). The diversity and the number of microorganisms changes considerably from the mouth to the large intestine. This is influenced by environmental conditions like pH, transit time and nutrient availability (Lin *et al*., 2014). The gut microbiome consist of beneficial (mutualistic), commensal and harmful microorganisms. When the beneficial species are predominant, the gut is in a state of normobiosis which means that there is a healthy gut composition. If the harmful species are predominant, the gut is in a state of dysbiosis, which means an unhealthy gut microbial composition for the host. The diversity and composition of the microbiome is affected by several factors which include type of feeding in early infancy, diet, medication, and other lifestyle factors of the host (Tojo *et al.*, 2014). This means that the microbe composition differs among individuals. However, certain species are found consistently in all people, such as *Faecalibacterium prausnitzii*, *Roseburia intestinalis*, *Bacteroides uniformis*, several *bifidobacteria* and *lactobacilli* (Tojo *et al.*, 2014). The microorganisms inhabit the human gut from birth and their numbers reach the maximum levels in the early adulthood, after which they decrease with age (Vos *et al*., 2012).

The human gut microbiome interacts with the host through the immune system, intestinal cells and mucus (Vos *et al*., 2012). The most important functions of the human gut microbiome are divided in metabolic, protective and structural functions (see *Figure 6*) (Grenham *et al*., 2011).

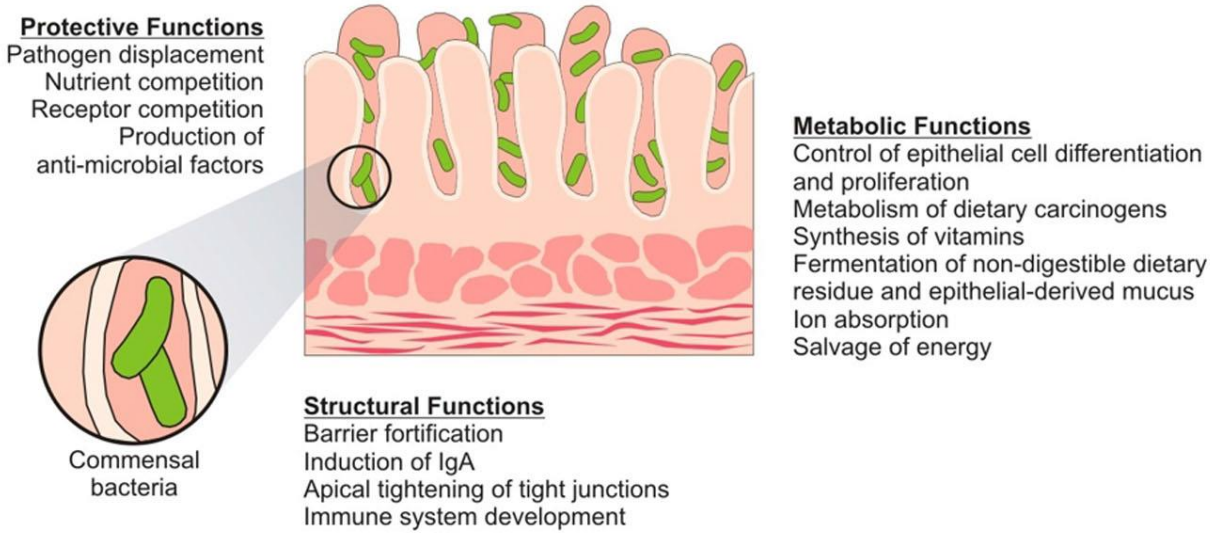


Figure 6. Functions of the gut microbiome. Source: Grenham *et al*., 2011.

Metabolic functions

The interaction of the gut microbiome with the host in relation to food are depicted in *Figure 7.* Part of the ingested food is digested and taken up directly by the host. Indigestible food components, like indigestible carbohydrates, are fermented by bacteria in the large intestine. The main metabolites produced are short chain fatty acids (SCFAs), which can be absorbed by the host. Bacteria that are frequently involved in SCFA production are for example *Ruminococcus, Lactobacillus, Bifidobacterium* and *Clostridium*. In order to do this, these bacteria possess a large diversity of enzymes e.g. sulfatases, galactosidases and glucuronidases. Beside SCFAs other metabolites are also produced e.g. formic acid, acetic acid, phenol, CO_2 and many sulfur containing compounds (Lin *et al*., 2014).

Depending on the fermentation type and products, the microbiome can promote healthy or impaired gut functions. An example of a healthy gut function is the fermentation of carbohydrates, which is beneficial for the host. The main products of carbohydrate fermentation are the SCFAs acetate, propionate and butyrate. These SCFAs have nutritional, regulatory and immune-modulatory properties (Lin *et al*., 2014). An example of impaired gut function caused by microorganisms is the production of trimethylamine and trimethylamine-N-oxide by bacteria that ferment the remainders of red meat. These compounds are linked to cardiovascular disease. Additionally, the bacterial fermentation of red meat can cause colorectal cancer. This is due to the fermentation of undigested proteins and the production of bacterial metabolites, which affect the function and renewal of the epithelial cells (Lin *et al*., 2014).

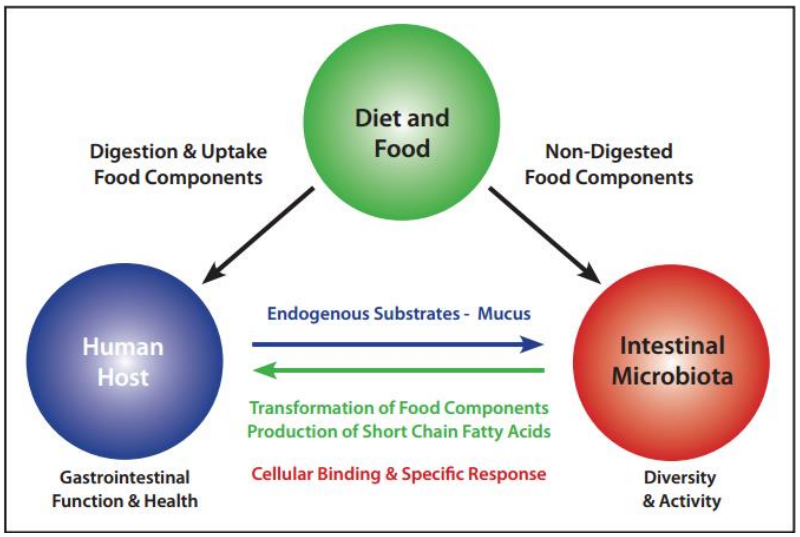


Figure 7. Interactions of the intestinal microbiome (microbiota) with the human host. Source: Vos *et al.*, 2012.

Protective functions

Intestinal antimicrobial proteins (AMPs), like certain members of the α- and β-defensins, are natural antibiotic proteins produced by and to protect the epithelial cellular surfaces such as the intestine, skin, respiratory tract and reproductive tract. These natural antibiotics are absent from the luminal content of the intestine but are located in the mucus layer. Some AMPs require bacterial molecular patterns for their expression. In the same way, Toll-like receptors (TLRs) present in the cells of innate immune system respond to certain patterns of ligands from commensal bacteria like polysaccharide A, lipopolysaccharide and lipoteichoic acid. This starts a signalling cascade that increases cytokine production and T-cell activation. This makes the buffering function of the mucus layer a chemical barrier against bacteria (Grenham *et al.*, 2011; Gallo and Hooper, 2012). Additionally, beneficial microorganisms protect against pathogen colonization by competition for nutrients and co-aggregation (see section *1.2.4 Probiotics, prebiotics and postbiotics* for further details).

Structural functions

The morphological influences of the microbiome in the gut can be analysed using germ-free (GF) animals; these are maintained sterile from the uterine environment by surgical delivery, eliminating the birthing and post-natal colonization steps. Studies have shown that an enlarged cecum, reduced intestinal surface area and smaller villous areas are some consequences of a lack of microbes (Grenham *et al.*, 2011).

Commensal bacteria can activate intraepithelial cells, which is essential for many functions such as epithelial cell proliferation, intestinal barrier integrity (for example the mucus layer separating the gut lumen from the endothelial cells), modulation of the innate immunity and the composition and maturation of immune cells (Lin *et al.*, 2014; Tojo *et al.*, 2014).

1.2.4 Probiotics, prebiotics and postbiotics

Mechanisms of gut microbiome restoration

The human body is sterile before birth (Reid *et al.*, 2011). After birth, the body is colonized by many different microorganisms. Some come from the mother and others from the environment. A healthy host has a balance between pathogenic and non-pathogenic microorganisms. When the balance is

disturbed, an infection or inflammation will be the result. Usually, nonspecific antibiotics are used to clear the infection. However, the antibiotics not only kill pathogenic microbes but also commensal and mutualistic microbes. Furthermore, a substantial delay in restoration of the healthy microbiome can occur after the use of antibiotics. Moreover, in some cases no antibiotics need to be used because the body can resolve the infection on its own. This process can be supported by probiotics. For example, *lactobacilli* have several mechanisms by which they affect pathogenic microorganisms. These mechanisms are: co-aggregation, production of biosurfactants, bacteriocin and H_2O_2 production, signalling to other bacteria, competitive exclusion, immunomodulation and modulation of tight junctions (Reid et al. 2011). An overview of these mechanisms is presented in *Figure 8*, and they are further described below. Although the mechanisms are explained for *lactobacilli*, they are also relevant for other (probiotic) bacteria. Moreover, some of these mechanisms, such as co-aggregation, competitive exclusion and immunomodulation, are also relevant in the restoration and maintenance of the skin microbiome.

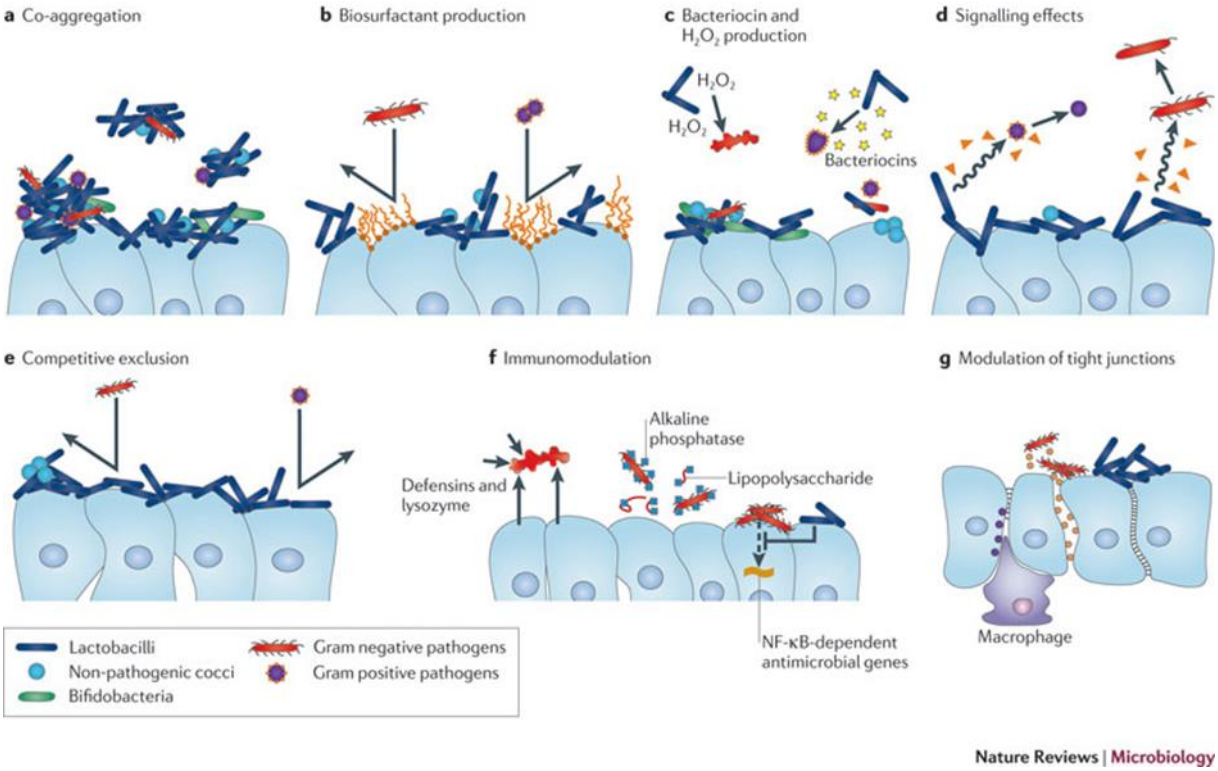


Figure 8. The possible mechanisms whereby probiotics can help restore and maintain the gut microbiome. Source: Reid et al. 2011. Some of these mechanisms are also relevant to the skin microbiome, such as co-aggregation, competitive exclusion and immunomodulation.

Co-aggregation

The ability of pathogens to infest the host is hindered by co-aggregation (biofilm formation) by *lactobacilli*. This means that *lactobacilli* and pathogens clump together, which disarms the pathogens. When the non-pathogens are killed, for example by the usage of antibiotics, the biofilm will be broken which leads to an opportunity for pathogens to infest the host (Alexander et al., 2003)

Biosurfactants production

Lactobacilli can produce biosurfactants. These consist of proteins, lipids and carbohydrates that can prevent adhesion of pathogens to the mucosal surface (Banat et al., 2010).

Bacteriocin and H_2O_2 production

Lactobacilli can also produce bacteriocins and hydrogen peroxide (H_2O_2) to inhibit or kill pathogens (Reid et al. 2011). Bacteriocins are produced by *lactobacilli* that selectively target competing bacterial strains. They interfere with the cell wall structure by forming pores in the target bacterial membrane and render the membrane permeable, thus killing the competing bacteria. The proteins also interfere with the biosynthesis of molecules (Hasper et al. 2006). H_2O_2 is a powerful oxidizing agent. It can kill pathogens through formation of free radicals. Free radicals are atoms or molecules that have at least one unpaired electron and is therefore unstable and highly reactive. The free radicals react with e.g. proteins, nucleic acids (Xu et al., 2008).

Signalling effects

Signalling between *lactobacilli* and pathogenic bacteria can result in a downregulation of toxin production. The reduced toxin production results in a reduction of inflammation and damage in the host (Laughton et al., 2006).

Competitive exclusion

In order to have a stable microbiome of indigenous bacteria, the commensals must have some features that strengthen their ability to colonize the host. For example, *lactobacilli* produce multiple capsular polysaccharides that are essential for colonization in the gut (Liu et al,. 2008). These polysaccharides help to exclude pathogens and restore homeostasis. In addition, competition for nutrients and surface receptors can exclude non-pathogenic bacteria from host surfaces (Aas et al., 2003).

Immunomodulation

The immune system and the microorganisms can regulate each other via three ways:

1. Antimicrobial factors, such as defensins, haemocidins and lysozymes, can kill pathogens and therefore suppress their growth. This results in the restoration of the microbial equilibrium (Pamer *et al*., 2007).
2. Alkaline phosphatases can bind to the lipopolysaccharides on the outside of the gram-negative bacteria. This negates the toxicity of these lipopolysaccharides and reduces the immune response (Reid *et al*. 2011).
3. Nuclear factor-κB (NF-κB) is a protein complex that controls the transcription of DNA of immune related genes. Commensal bacteria can downregulate the immune response (Ryu *et al*., 2008). *Lactobacilli* could possibly downregulate NF-κB and suppress the immune system. However this has not yet been confirmed.

Modulation of tight junction

The integrity of the epithelial lining in the gut, mouth and vagina plays an important role in maintaining health. When the lining is disrupted, microorganisms on the outer surface can enter the tissue or bloodstream, which will cause disease. There are two ways in which *lactobacilli* affect the tight junctions:

1. The tight junction proteins could possibly be upregulated by *lactobacilli* (Karczewski *et al*, 2010).
2. The *lactobacilli* can coat the surface of the gut. This coating can protect the tight junctions (Reid *et al*., 2011).

If there is damage to the tight junctions a host response is induced. For example a macrophage can enter and further induce local damage when attacking the invaded pathogens with antimicrobial peptides (Reid *et al*., 2011).

The mechanisms described are not only utilized by *lactobacilli*, but also by other microbes. Furthermore, many of the mechanisms described are not only important in the gut, but are likely also important in skin microbiome symbiosis.

Probiotics

As defined by the World Health Organization, probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). Therefore, oral probiotics can be regarded as functional foods: foods that enhance health beyond their basic nutritional value. Probiotics mainly modulate the host immune system and this research field has recently gained more interest (Soccol *et al*., 2010). Some other benefits of probiotics are the production of vitamins, antioxidants and SCFAs and inhibiting the growth of pathogens (Lin *et al*., 2014), see also section *1.2.3 Gut microbiome*. A list of established and proposed health effects of probiotics can be found in *Table 1*, along with their current state of scientific proof, in order of decreasing validity.

Table 1. Established and proposed oral probiotic health effects (Adapted from Vrese and Schrezenmeir, 2008). On the left, the established and proposed health effects are displayed, on the right the current state of scientific proof, in order of decreasing validity.

Effect of probiotics	Validity of scientific proof
Prevention and/or reduction of duration and complaints of rotavirus-induced diarrhea	Effect well-established by clinical studies and accepted by the scientific community
Alleviations of complaints due to lactose intolerance	
Modulation of the autochtonous (usually intestinal) microbiome	Well-established effect but because of methodological difficulties, the correlation with true health effects is unclear
Immunomodulation and/or regulation	
Reduction of the concentration of cancer promoting enzymes	
Prevention or alleviation of allergies and atopic diseases	Effects are observed in certain target groups. However more studies are necessary
Beneficial effects on microbial aberrancies, for instance, Inflammatory diseases of the gastrointestinal tract and bacterial overgrowth	
Treatment of urogenital infections	
Prevention of respiratory tract infections	
Cancer prevention	Insufficient clinical and epidemiological data the effects cannot be considered scientifically proven
Normalizing of passing stool	
Prevention or therapy of ischemic heart diseases	
Hypocholesterolemic effect	Reliable effects are not proven at all
Improvement of mineral absorption	
Caries prevention	

Many probiotic bacteria are lactic acid producing bacteria. Lactic acid producing bacteria are gram-positive microorganisms that are capable of fermenting carbohydrates and higher alcohols and of course produce lactic acid. These bacteria range from strictly anaerobic to aerobic (including facultative anaerobic) and do not form spores. They include Actinomycetes (>55% G+C content) and

Clostridium (<55% G+C content) and can be divided into homofermentative (lactid acid as primary metabolite) and heterofermentative (multiple primary metabolites: lactate, CO_2, ethanol, acetate) (Stiles and Holzapfel, 1997; Klein *et al*., 1998; Ying and Demarchi, 2013).

Some of the genera most commonly used as probiotics are: *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Streptococcus* and *Bifidobacterium*. From these, *Lactobacillus* and *Bifidobacterium* are the most commonly used in commercial preparations (Vrese and Schrezenmeir, 2008; Ying and Demarchi, 2013). However, some other microorganisms like *Escherichia coli* and certain yeasts can also be used as probiotics (Ying and Demarchi, 2013). *Lactobacillus* is fermentative, acidophilic, and aerotolerant or anaerobic. The environment they prefer is rich in carbohydrate containing substrates like in mucosal membranes, plants, plant material, sewage, fermented (milk) products or food waste (Vrese and Schrezenmeir, 2008). *Bifidobacteria* are a major part of the normal microbiome of humans. They occur soon after birth and their amount decreases with age. These bacteria are strictly anaerobic, do not produce gas, are gram positive and are present in dental caries, faeces and the vagina (Vrese and Schrezenmeir, 2008; Soccol *et al*., 2010).

The microorganisms used for human health usually have the following characteristics: they are bile acid tolerant, resistant to low pH, from a human origin, non-pathogenic, have a good survivability during processing and a good shelf-life. It is also important that probiotics have evidence of beneficial health effects (Ying and Demarchi, 2013). Furthermore, probiotics must be free of a contamination with pathogens or toxins. Moreover the bacteria should be able to adhere and survive in the intestine for a longer period of time (Vrese and Schrezenmeir, 2008).

Many studies investigating possible mechanisms by which oral probiotics exert their effects have been published in recent years. *Table 2* describes a number of these studies, the strains investigated and the proposed underlying mechanism.

Table 2. Examples of proven effects of probiotics.

Genus	Species	Disease/ Symptom	Mechanism	Reference
Lacto-bacillus	*Lactobacillus rhamnosus GG* (LGG)	Diarrhoea	Excretion of biosurfactants, including lactic acid, bacteriocins, organic acids and hydrogen peroxide to limit the pathogen growth.	Guandalini *et al*., 2000
	LGG	Inflammatory bowel disease (IBD)	Inhibition of adherence of pathogenic bacteria to epithelial receptors *in vitro* by inducing intestinal mucin gene expression; antagonizing pathogens through competition for pathogen receptor sites, nutrients, or production of antimicrobial compounds.	Mack *et al*., 1999
	Lactobacillus casei (DN114001)	Irritable Bowel Syndrome (IBS)	Induction of mucosal immune stimulation, thereby reinforcing the non-specific barrier and modulating the innate immune response in the gut, maintaining intestinal homeostasis.	Maldonado *et al*., 2009
	Lactobacillus salivarius	Chronic gastritis, peptic ulcers, gastric cancer	Inhibition of colonization and release of IL-8 in gnotobiotic mice inoculated with *H. pylori*.	Kabir *et al*., 1997
	Lactobacillus casei	Influenza virus	Stimulate the cell immune response by induction of IL-12, IFNγ and TNFα.	Hori *et al*., 2001

Bifido-bacterium	*Bifidobacterium bifidum str.* BGN4	Eczema	Help creating a more eubiotic state in mother and newborn, leading to immune modulation and reduced risk of eczema.	Kim, J. Y. *et al*., 2010
VSL#3*		IBD	Improving mucosal production of IL-10 and reducing secretion of TNFα and IFNγ.	Gionchetti *et al*., 2003
		IBS	Relief of abdominal pain, bloating, flatulence and urgency. Analysis of gastrointestinal transit, satisfactory relief and stool and symptom scores.	Kim *et al*., 2003
Saccharo-myces	*Saccharomyces boulardii*	Toxin A produced by Clostridium difficile	Preventing intestinal injury and inflammation by inhibiting the activation of extracellular signal-regulated ½ (ERK ½) and mitogen activated protein (MAP) kinases, thus modulating host signaling pathways. Increase in specific anti-toxin A levels.	Chen *et al*., 2006 Qamar *et al*., 2001

* VSL#3 is a potent probiotic medical food that delivers the highest available concentration of beneficial live bacteria of any probiotic in the world. It contains 1 strain of *Streptococcus*, 3 strains of *bifidobacterium*, and 4 strains of *lactobacillus* and maltose and silicon dioxide. Those specific strains are *Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei, Lactobacillus delbrueckii* subsp. Bulgaricus (Gionchetti *et al*., 2003).

The physiological effects of probiotics can differ within closely related strains, therefore proof of their beneficial effects is only valid for specific bacterial strains. Other factors influencing the physiological effects include the amount and duration of administration, composition (for example, the combination of two or more strains) and the type of vehicle in which the bacteria are ingested. The effects of probiotics also vary with age, gender, health and diet. These variations make the transferability of the results among different groups of people difficult (Vrese and Schrezenmeir, 2008).

Prebiotics

Prebiotics are nutrients, food ingredients or growth substrates that modulate and benefit the microbiome already present in the host. These can be used to achieve homeostasis by promoting growth of the good bacteria. As Gibson and Roberfroid (1995) stated, a prebiotic is “A non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and activity of a limited number of bacteria in the colon, and thus improves host health”. The growth of e.g. *bifidobacteria* and *lactobacilli* are preferred over potentially pathogenic bacteria (Lin *et al*., 2014).

Two main groups of prebiotics according to their chemical structure are: Inulin-type fructants (ITF) (fructo-, oligo- and polysaccharides) and galacto-oligosaccharides (GOS). These cannot be digested by the host itself and have chains consisting of 3 to 10 monosaccharides. However, they can be fermented by specific bacteria. Prebiotics must fulfil the following criteria:

- Non-digestible or not absorbed by the host and resistant to gastric acid and enzyme digestion
- Fermented by intestinal microbiome
- Selective stimulation of bacterial growth

These can be found in dietary fibres mainly by *Bifidobacterium* (Vrese & Schrezenmeir, 2008). They are metabolized into short chain fatty acids, lactid acid, hydrogen, methane and CO_2. These products can be taken up by the host and have the following positive effects:

- Growth promotion of protective bacteria
- Inhibition of potentially pathogenic microorganisms
- Stabilization of intestinal environment (lowering pH and release of short chain organic acids (Vrese & Schrezenmeir, 2008).

Several studies have investigated the mechanisms by which prebiotics exert their effects. In *Table 3* a number of prebiotic substances, the disease or symptom they affect and the mechanism by which they are proposed to exert their effects are summarized.

Table 3. Examples of proven effects of prebiotics.

Disease/ Symptom	Prebiotics	Mechanisms	Reference
Constipation	Lactulose, FOS, GOS	Osmotic effect and modulation of indigenous microflora.	G.R. Gibson *et al*. 2004
Hepatic encephalopathy	Lactulose, Lactilol	Bacterial incorporation of nitrogen and acidification of the colonic environment which in turn reduces the breakdown of nitrogen-containing compounds to ammonia and other potential cerebral toxins.	Delzenne, 2003 Marteau & Boutron-Ruault, 2002
IBD	Inuline, FOS, GOS	Regulating immune responses to commensal and pathogenic bacteria.	Marteau & Boutron-Ruault, 2002 Cherbut *et al*, 2003
Cholesterol gallstones prevention	FOS, IMO, GOS, palatinose condensate, raffinose	Stimulating the growth of *bifidobacteria* in vitro and in vivo.	Mitsuoka *et al*., 1987
Prevention of infections of intestinal origin	Oligosaccharides	Contributing to a greater resistance to infection. Most of *Bifidobacterium* species have scavenging function.	Kohmoto, *et al*. 1988

FOS: fructo-oligosaccharides, GOS: galacto-oligosaccharides, IMO: Isomalto-oligosaccharide

Postbiotics

Probiotics produce and secrete molecules that can have beneficial effects on the host. These products are called postbiotics and are for example SCFAs, acetic acid and anti-inflammatory molecules. Compared to probiotics, postbiotics could be safe alternatives for immunocompromised patients and an environment with heavy pro-inflammatory components (Hamer *et al.*, 2008; Tsilingiri and Rescigno, 2013; Lin *et al*., 2014).

2. METHODOLOGY

An overview of the approach we followed during this project is depicted in *Figure 9*. First, research was divided into three parts: i) information from the literature about the main topics involved in project, that is, skin conditions, healthy skin and probiotics; ii) pinpointing of potential mechanisms that could be targeted by probiotics in both healthy and diseased skin; iii) market study, in which current skin care products claiming to contain probiotics are summarized along with a short description of their mechanisms (if known) and effects. Secondly, integrating these three parts allowed us to have a complete picture of the case; based on this the advice was formulated. The final step was decided depending on the advice: if the use of probiotics for topical skin care products was recommended, then recommendations for further research were created; if not, alternative solutions were proposed. In this way, the outcome of the advice led us to the recommendations for further research. The previous steps were performed following a previously designed decision tree depicted in *Figure 10.*

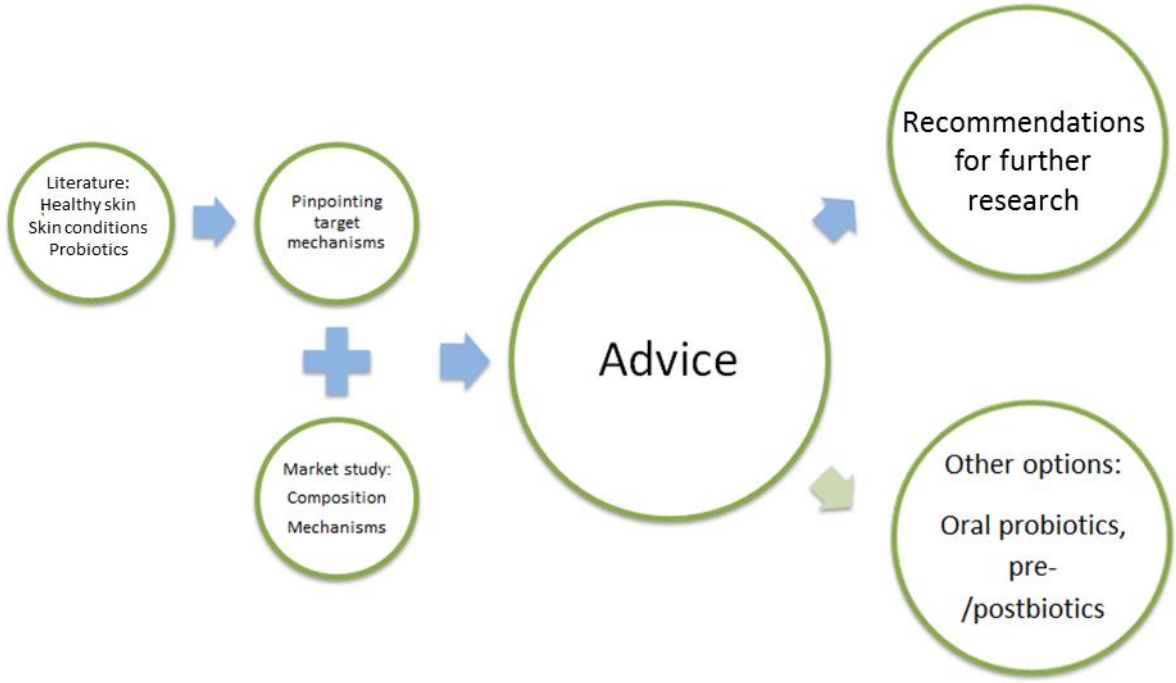


Figure 9. Research Process and Methodology. The study was divided into three parts: literature overview of the main topics, localization of target mechanism and market study; all together this information leads to a final advice. If the advice is in favor of probiotics in skin care products, the final step is to write recommendations for further research; if the advice is negative, the final step is to look at other options such as pre- or postbiotics. The pathway that this project took is indicated with blue arrows.

i) Literature research main topics

Each topic from the literature search (skin conditions, healthy skin and probiotics) was further divided into subsections. The topic 'skin conditions' was limited to acne, rosacea and atopic dermatitis. For each of the selected skin conditions at least the symptoms, underlying mechanisms and current treatments were described. The topic 'healthy skin' was further divided into general information about the skin and skin microbiome. Finally, the 'probiotics' part was subdivided into gut microbiome, host-microbe interactions and general information about probiotics, prebiotics and postbiotics. The information on probiotics can be found in section *1 Introduction* (see *Figure 10*).

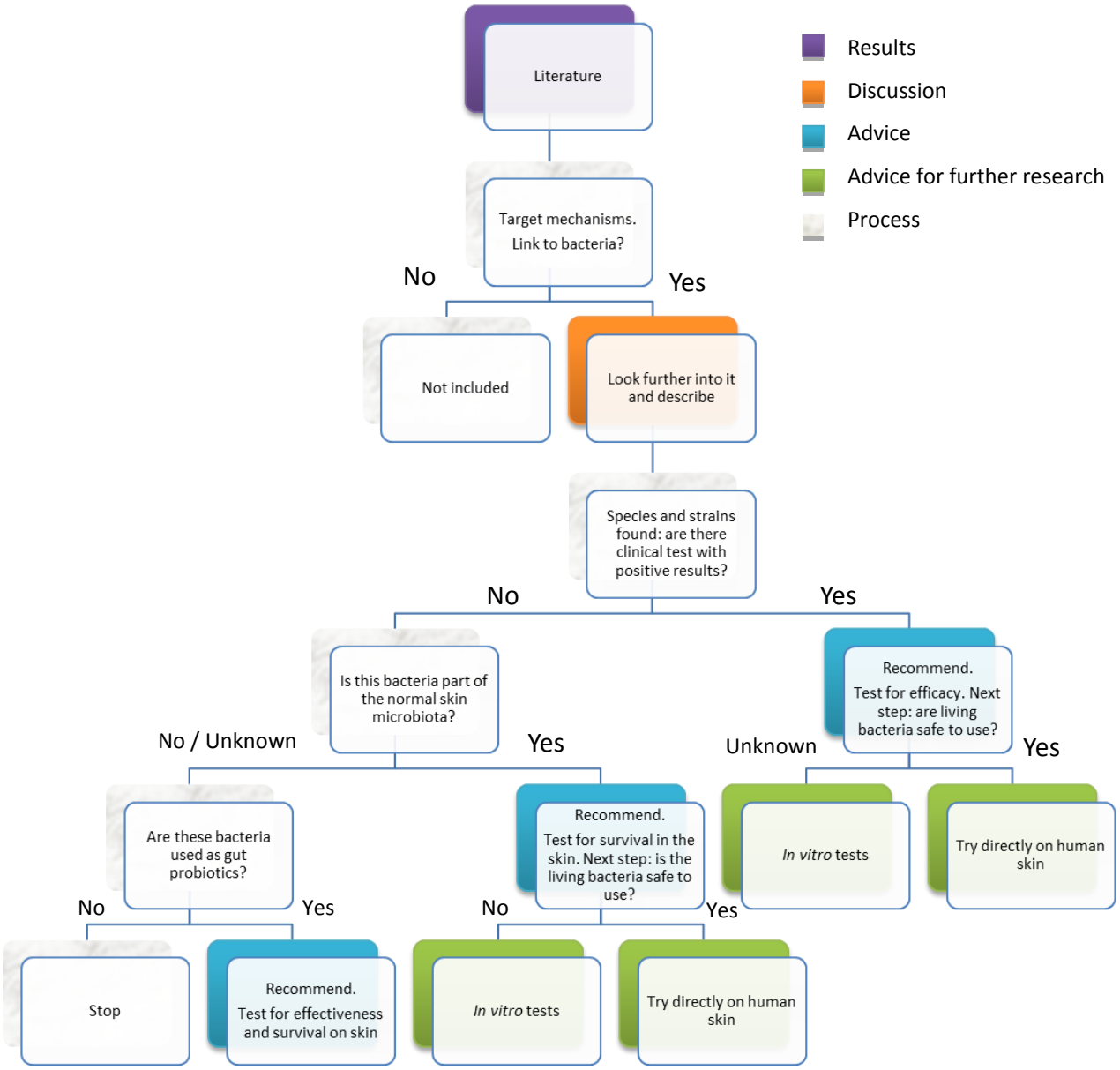


Figure 10. Decision tree. Here it is shown the criteria and steps followed to pinpoint target mechanisms and to select promising probiotics species. It is indicates the information included in each part of the report.

The information about healthy skin and the skin conditions can be found under section *3 Results*. All information was gathered from the databases PubMed and Google Scholar; the articles were from peer-reviewed journals. Combinations of various keywords were used. A list of keywords used can be found in the *Appendix.*

ii) *Pinpointing target mechanisms*

From the information found in part *i*, we selected mechanisms that seemed most promising to target using probiotics in healthy skin and in each skin condition. Potential probiotic or bacterial strains

influencing selected mechanisms were discussed. This information can be found under section *4 Discussion* (see also *Figure 10*). Literature research was done as described previously.

iii) Market study

Skin care products that are currently available on the market and that claim to contain probiotics were summarized in a table. For each product the name of the product, the producer, the probiotic ingredients (if known), the mechanism of action as claimed by the producer, the mode of application and the relevant references were indicated. This was done by two means: 1) internet research through the search engine Google, using combinations of the key words (*see Appendix*) "rosacea", "acne", "atopic dermatitis" and "healthy skin" with "probiotics", "treatment", "topical" and related words. The search was done in Chinese, English and Dutch. 2) Research directly in health food shops, cosmetics and beauty stores and departments as well as pharmacies and general stores found in the main street at the centre of Wageningen, The Netherlands. The ingredients according to the packages were examined for probiotics and shop staff was asked if they sell "skin creams" or "beauty treatments" containing probiotics. This information can be found under section *3 Results*. The discussion of this part can be found under section *4 Discussion.*

iv) Advice

An advice was formulated based on the most promising strains that were found. For each skin condition and for healthy skin, we advise if using probiotics to treat them is recommended or not. If we give a positive advice, we recommend further research that can be done to verify the effects of the most promising probiotics. If we give a negative advice, potential alternatives are given. This information can be found under section *5 Conclusions and Advice* (see *Figure 10*).

v) Recommendations for further research

Recommendations for further research were written for the probiotics that we advise in the previous section. Depending on the current state of knowledge of these probiotics, further steps were recommended to confirm their supposed effects (see *Figure 10*).

3. RESULTS

3.1 Healthy skin

3.1.1 The skin

The skin is the largest organ of the human body. It provides a physical barrier that protects the body from toxic substances and potential assaults by foreign organisms, while keeping moisture and nutrients inside the body. Moreover, the skin is an interface with the outside environment and is colonized by many different microorganisms as well as mites (Grice and Segre, 2011).

The skin barrier consists of several layers. The main layer affecting the skin microbiome is the epidermis (see *Figure 11*) (Sanford and Gallo, 2013).

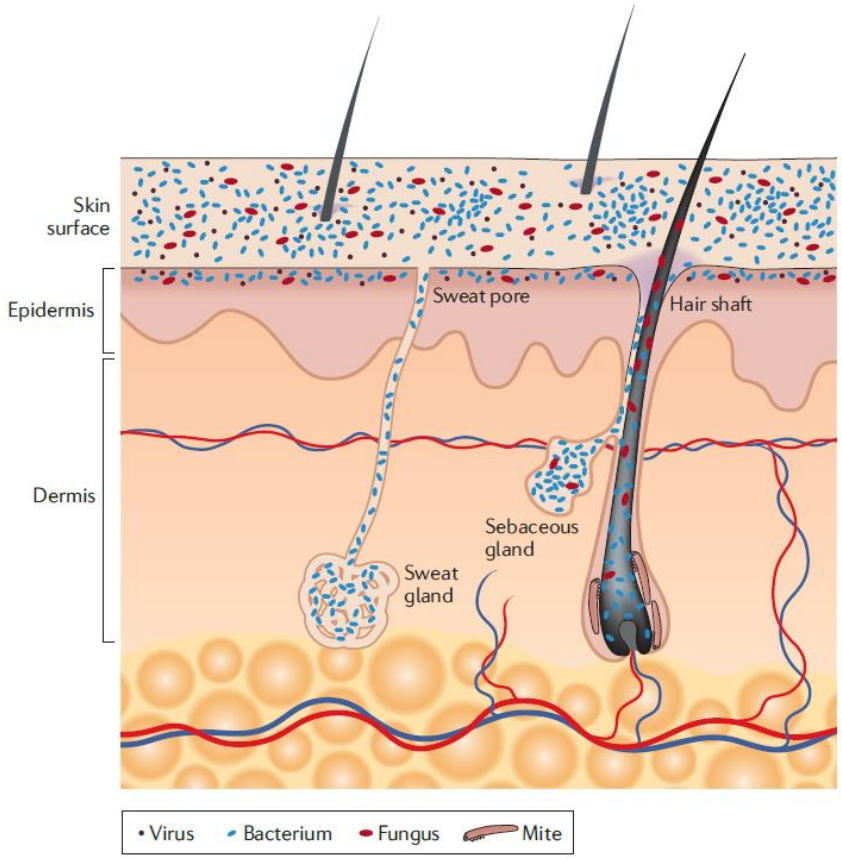


Figure 11. Schematic cross-section of skin histology, including localization of the different types of microorganisms, namely viruses, bacteria and fungi, as well as mites. Bacteria are present on the skin surface and also reside in the hair and glands. Source: Grice and Segre, 2011.

The epidermis protects the body from the outside world. The outer layer of the skin is the cornified layer, also called the *stratum corneum* (see *Figure 12*). The differentiation of the keratinocytes in the epidermis needs to be tightly regulated to maintain homeostasis (Candi *et al.,* 2005).

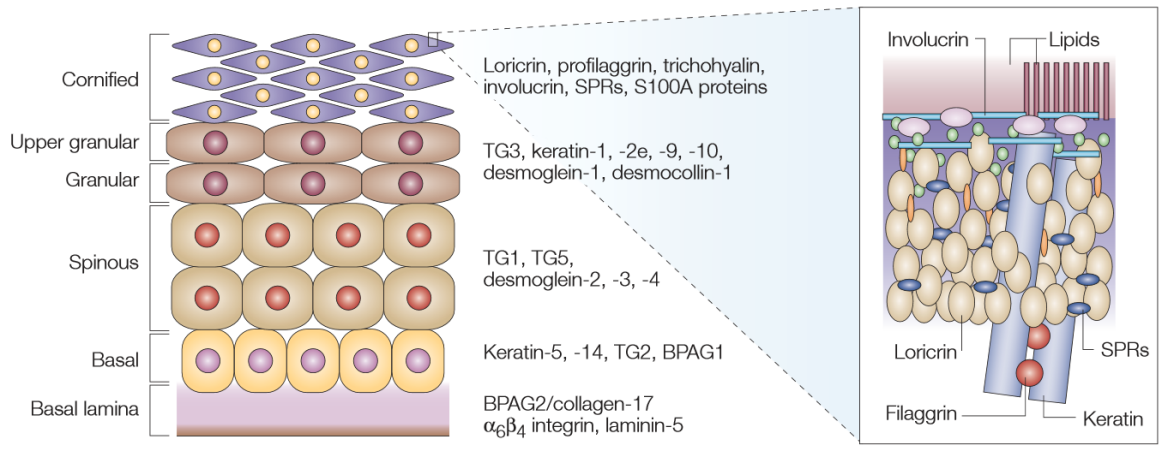


Figure 12. A schematic overview of the epidermis. Filaggrin can be found in the outermost layer, the cornified layer or *stratum corneum*, where it is essential for arranging the structure. The cornified layer consists of the cornified and lipid envelope. The cornified envelope which is imbedded in the lipid envelope. Ceramides can be found in the lipid envelope surrounding the cornified envelope. Source: Candi *et al.*, 2005.

The cornified layer consists of dead corneocytes and is constantly resupplied with living epidermal keratinocytes which terminally differentiate into corneocytes (Reichert *et al.,* 1993). The skin is a continuously self-renewing organ, with corneocytes constantly being shed from its surface. The migration from the basal layer and differentiation into corneocytes takes approximately 4 weeks (Grice and Segre, 2011). One of the most important parts of this differentiation is the formation of a stable protein envelope which is called the cornified envelope (CE) (Reichert *et al.,* 1993). This envelope is a 15 nm thick layer of insoluble proteins (Steinert and Marekov, 1995). The CE is essential for the water barrier function of the epidermis (Kalinin *et al.,* 2002). The process of forming the CE is highly organized in both space and time and begins with the migration of keratinocytes from the basal layer all the way to the terminal differentiation in the cornified layer (Candi *et al.,* 2005). A vital protein in the formation of the cornified envelope is filaggrin (Palmer *et al.,* 2006).

The CE is embedded in a lipid envelope. This lipid envelope consists mostly of ceramides, cholesterol, cholesterol esters and fatty acids. Basic ceramides are produced by conjugating a sphingoid base and a fatty acid via an amide bond. One such sphingoid base is sphingosine (Meckfessel and Brandt, 2014). The lipid envelope contributes to the flexibility and barrier function of the CE. An important factor to keep the cornified layer moisturized is the Natural Moisterizing Factor (NMF), which is found exclusively in the cornified layer and can be make up as much as 10% of the dry weight of the cells. NMF is a mixture of amino acids, salts and derivatives of amino acids, which can maintain levels of moisture when exposed to low humidities. It is retained inside the corneocytes by lipids from the lipid envelope (Rawlings *et al.,* 1994). So the lipid envelope is important for the barrier function of the CE and reducing the water loss via among others retaining NMF in the corneocytes.

The skin contains many invaginations, including sweat glands, hair follicles and sebaceous glands. There are two types of sweat glands: eccrine and apocrine. Eccrine sweat glands are distributed across almost the entire skin surface and are crucial in thermoregulation. They secrete sweat that is mainly composed of water and salt. Evaporation of the water allows the body to cool, whereas the salt helps acidify the skin, thereby limiting the colonization and growth of microorganisms. (Grice and Segre, 2011; Sanford and Gallo, 2013) In addition, eccrine sweat glands constitutively excrete several antimicrobial peptides (Sanford and Gallo, 2013). Apocrine sweat glands are found primarily in the arm pit (axillary vault), nipple and genitoanal region. They are already present at birth, but become

active during puberty. In response to adrenaline, these glands produce milky, oily, odourless mixtures of proteins, lipids and steroids. Degradation of apocrine gland secretions by bacteria is responsible for the characteristic smell of sweat. (Grice and Segre, 2011; Sanford and Gallo, 2013)

Another type of gland found in the skin is the sebaceous gland. Sebaceous glands are connected to hair follicles and together form the pilosebaceous unit. These glands secrete sebum: a hydrophobic coating that protects and lubricates skin and hair and provides an antimicrobial shield. (Grice and Segre, 2011; Sanford and Gallo, 2013)

3.1.2 Microbiome on the skin

Our skin is colonized by a large number of microorganisms: 1 cm^2 of skin is estimated to contain 1 million bacteria, from the surface down to the appendages like hair follicles and glands (Grice *et al.*, 2008). In general, the skin is dry, cool and acidic. (Grice and Segre, 2011)

The skin spans an area of 1.8 m^2 and with its many folds, invaginations (infolding of one part within another part of a structure) and specialized niches, it supports a wide range of microorganisms. The different chemical and physical properties of different skin areas select for unique groups of microorganisms adapted to the niche they inhabit. Thus, the composition of the skin flora is not uniform: different regions of the skin support distinct groups of microorganisms, see *Figure 13*. This is due to differences in skin topography (see also *Figure 14*), host factors and environmental factors (Grice and Segre, 2011).

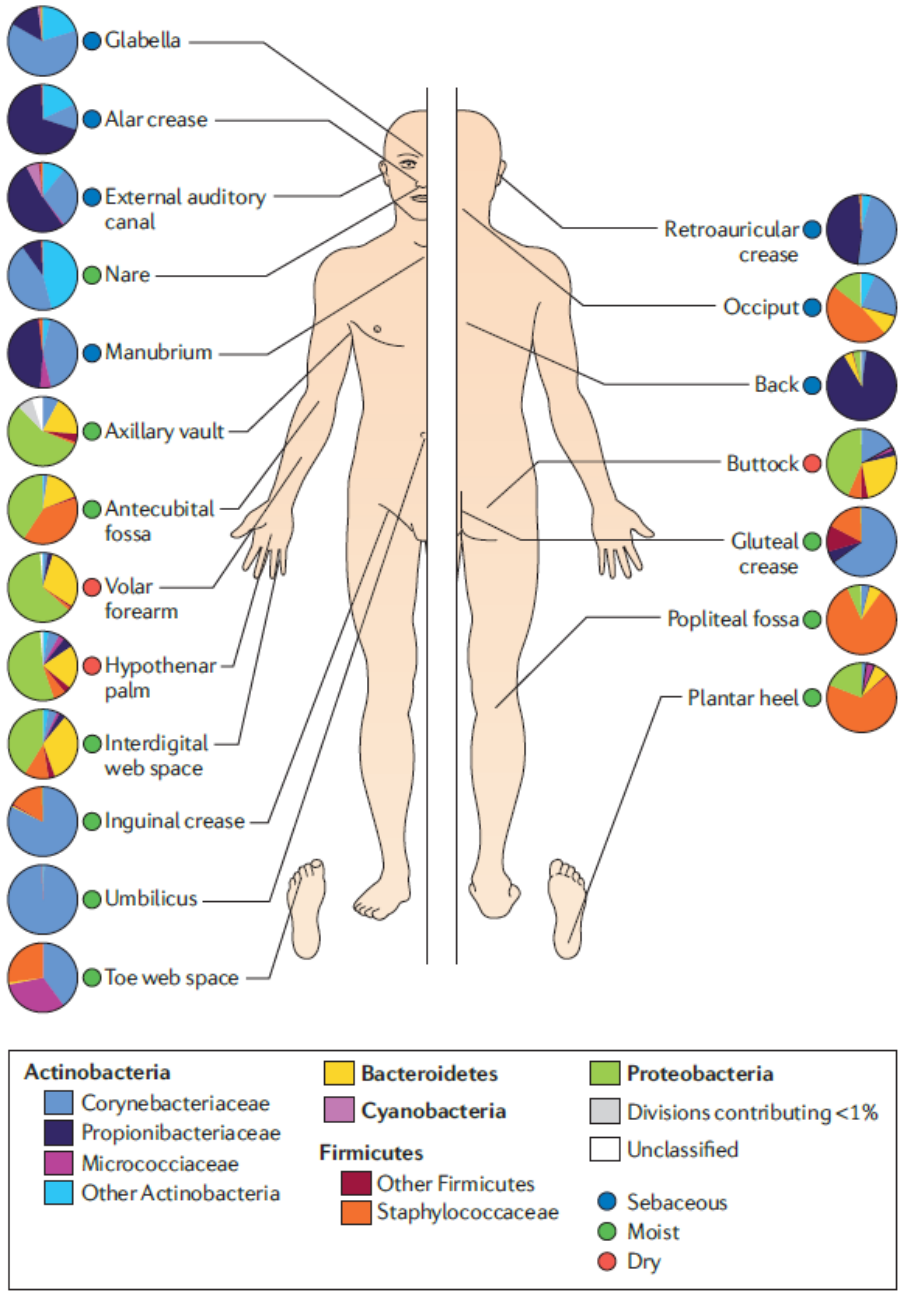


Figure 13. Topographical distribution of bacterial phyla (bold) and families on skin sites. The areas are subdivided into sebaceous (blue circle), moist (green circle) and dry (red circle) areas. Source: Grice and Segre, 2011.

3.1.3 Photoprotection by oral probiotics

A few clinical studies and one *in vitro* and animal study have investigated the effects of probiotics on photoprotection. However, these all used oral as opposed to topical probiotics. Therefore, they are not discussed in section *4 Discussion*.

A study by a group of researchers from L'Oréal and Nestlé, Guéniche *et al*. (2009) conducted a randomized double-blind placebo-controlled clinical trial to investigate the effect of *Lactobacillus johnsonii* NCC 533 (La1) intake on the skin immune system after UV exposure. They found that UV exposure to twice 1.5 minimal erythemal dose (MED), the minimal dose that may produce sunburn, led to an earlier recovery of epidermal cells allostimulatory function. This means that intake of this probiotic led to quicker recovery from sunburn.

Bouilly-Gauthier *et al*. (2010) conducted clinical trials with healthy adult women (with skin type II-IV), in which they assessed the effects of a dietary supplement containing *Lactobacillus johnsonii* (La1) and carotenoids on UV protection. In the first clinical trial, subjects were exposed to nonextreme UV with a high UVA irradiance (UV daylight). Early markers for UV-induced skin damage were investigated through histology and immunohistochemistry. In the second clinical trial extreme solar radiation (UV-SSR) was used, and the MED was determined through clinical evaluation and chromametry. In the third clinical trial natural sunlight was used and dermatologists assessed the skin before and after exposure. The researchers found that a 10 week supplementation with La1 and carotenoids reduced early UV-induced skin damage.

A study by Kim *et al.* (2014) investigated the effect of oral administration of the probiotic *Lactobacillus plantarum* HY7714 on UVB-induced photoaging in human dermal fibroblasts (Hs68 cell line) and hairless mice. Photoaging is a type of skin damage that includes coarse and fine wrinkles, dryness, laxity (looseness), pigmentation and increased skin thickness. The collagen-degrading MMP-1 (matrix metalloproteinase-1) is induced by UVB and leads to collagen loss. The researchers found that treatment with this probiotic rescued procollagen expression by inhibiting UVB-induced MMP-1 expression in the Hs68 cells. Moreover, *L. plantarum* HY7714 clearly inhibited the number, depth and area of wrinkles in hairless mice, and decreased UVB-induced epidermal thickness.

Factors determining the skin microbiome

Skin topography greatly varies across the human body (see *Figure 14*). Some regions of the skin are partially occluded, for example between the toes or behind the ears, causing a higher temperature and humidity. This encourages the growth of microorganisms thriving in moist conditions like *Staphylococcus* spp. and *Corynebacterium* spp. (Grice, 2014). On the other hand, areas of the skin containing a relatively high density of sebaceous glands, such as the face, chest and back, will encourage growth of lipophilic microorganisms, e.g. *Propionibacterium* spp. and the fungal *Malassezia* spp. (Grice, 2014), as well as *Corynebacterium* spp. (Scharschmidt and Fischbach, 2013). Sebaceous areas tend to have a low diversity and are relatively stable over time compared to moist and dry skin sites (Grice, 2014). Furthermore, these sites also support colonization by the *Demodex* skin mites (Grice, 2014). Dry areas of the skin generally carry a greater microbial diversity and lesser bacterial load than sebaceous and moist areas (Grice, 2014). There seems to be less of a selective force at these sites, and they contain greater amounts of bacteria from the phyla Proteobacteria and Bacteroidetes compared to moist and sebaceous areas (Grice, 2014).

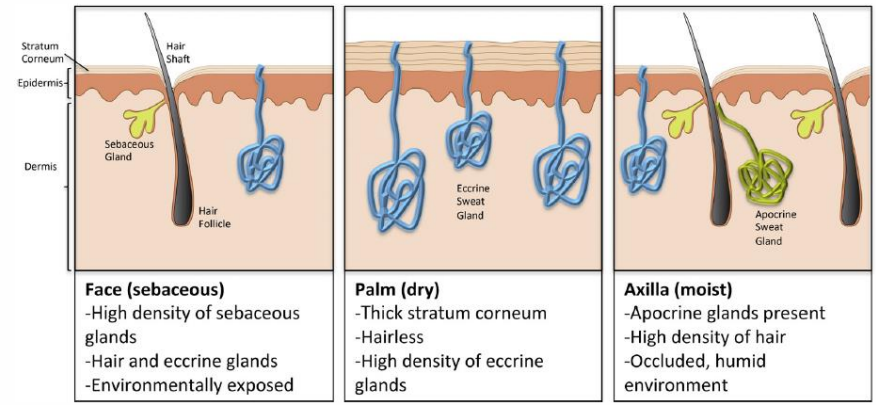


Figure 14. Representative illustrations of the three major categories of skin environments. Source: Sanford and Gallo, 2013.

Host factors such as genetic makeup, age, location, immune reactivity and sex also contribute to the skin microbiome (Grice and Segre, 2011; Kong and Segre, 2012). Furthermore, environmental factors like occupation, clothing choice, climate and antibiotic usage may modulate microbial colonization of the skin. The use of cosmetics, soaps, hygienic products and moisturizers may also contribute to variations in the skin microbiome (Grice and Segre, 2011; Kong and Segre, 2012).

The sweat and sebaceous glands are likely associated with their own unique microbiome (Kong and Segre, 2012). Because sebaceous glands are lipid-rich and relatively anoxic, they support the growth of facultative anaerobes such as *Propionibacterium acnes*, which is a common skin commensal. (Grice and Segre, 2011; Sanford and Gallo, 2013)

Common microbes on human skin

A study by Grice *et al.* (2009) investigated the topographical and temporal diversity of the human skin microbiome by characterizing samples taken from 20 different skin sites on 10 healthy volunteers. They found 19 different bacterial phyla, of which the four dominant phyla were Actinobacteria (51.8%), Firmicutes (24.4%), Proteobacteria (16.5%) and Bacteroidetes (6.3%). From the more than 200 identified sequences, *Corynebacteria* (Actinobacteria, 22.8%), *Propionibacteria* (Actinobacteria, 23.0%) and *Staphylococci* (Firmicutes; 16.8%) comprised the majority (Grice *et al.*, 2009). Most other species make up less than 1% of the total skin microbiota in a particular habitat. Many of these species have not been well studied, but could nonetheless be important parts of the skin flora (Chen and Tsao, 2013). However, as mentioned in *Factors determining the skin microbiome*, the abundance of each group of bacteria strongly depends on the characteristics of the skin area.

Below, a more detailed description is given on some types of microbes that commonly occur on healthy human skin and have been extensively researched.

Corynebacteria are gram-positive aerobes or facultative anaerobes that belong to the Actinobacteria. They are mainly found at moist or sebaceous skin sites. Members that are most found on the skin are *C. accolens*, *C. jeikeium*, *C. urealyticum*, *C. amycolatum*, *C. minutissimum* and *C. striatum*. *Corynebacteria* are lipophilic bacteria, and are actually lipid auxotrophs: they cannot produce their own lipids and are therefore dependent on lipids from the environment. They can utilize lipids as nutrients and to generate corynomycolic acids to coat their cell surface. Furthermore, they are halotolerant, which means that they can survive in high salt concentrations. This explains their ability to colonize skin sites high in eccrine sweat glands. Some *Corynebacteria* even rely on vitamins from sweat for survival (Scharschmidt and Fischbach, 2013). *Corynebacteria* are the main microorganisms that process the odourless eccrine, apocrine and sebaceous gland secretions, thereby producing volatile organic fatty acids and thioalcohols that are responsible for the bad odour of sweat from the axilla (armpit) (James *et al.*, 2012).

Propionibacteria are gram-positive anaerobic bacilli. *Propionibacteria* are mostly found in hair follicles, but can also be found on the skin surface (Scharschmidt and Fischbach, 2013). *Propionibacteria* hydrolyze triglycerides from the sebum into free fatty acids, which then acidify and soften the skin (Grice, 2014). The most well-known strain is *P. acnes.* This strain and *P. granulosum* live on sebaceous skin sites, while *P. avidum* is found in regions high in eccrine sweat. *P. acnes* possesses multiple lipases, which it probably uses to acquire nutrients from sebum, and proteases

that are capable of liberating arginine from skin proteins and is an important carbon and energy source. *P. acnes* also produces large amounts of porphyrins, but their function remains unclear (Scharschmidt and Fischbach, 2013). *P. acnes* will be discussed in further detail in the chapter about acne.

Staphylococci are gram-positive aerobes or facultative anaerobes that belong to the Firmicutes. The most prevalent strain is the commensal *S. epidermidis*, but other strains found on the skin include *S. hominis*, *S. capitis*, *S. saprophyticus*. *Staphylococci* are highly adaptable and are found mainly in moist areas, but also on dry, exposed skin sites like the palm and volar (on the side of the palm) forearm. Some facultative anaerobic *Staphylococcus* strains can survive in low oxygen habitats such as hair follicles besides the skin surface. *Staphylococci* use several strategies to survive on the skin. Firstly, they are halotolerant, just as *Corynebacteria*. They may even use the urea present in sweat as a carbon source. Secondly, they possess several adhesins, which may facilitate attachment to the skin, as well as proteases, which may help remodel the *stratum corneum* and liberate additional nutrients (Scharschmidt and Fischbach, 2013). Thirdly, sebaceous glands in the skin secrete several bactericidal factors, some of which are lipids. Many *S. epidermidis* strains produce an enzyme that inactivates these bactericidal lipids by esterifying them into cholesterol, thereby protecting the bacteria from the effects of the bactericidal lipids (Chamberlain and Brueggemann, 1997; Scharschmidt and Fischbach, 2013). Another member of the *Staphylococci* is *S. aureus. S. aureus* is a pathogen that can cause a wide variety of conditions. The appearance of drug-resistant *S. aureus* strains, such as methicillin-resistant *S. aureus* (MRSA), has made treatment of *S. aureus* infections difficult (Iwase *et al.*, 2010). Certain strains of *S. epidermidis* have been found to produce an extracellular serine protease called Esp (Dubin *et al.*, 2001). Esp inhibits biofilm formation, destroys pre-existing *S. aureus* biofilms and inhibits colonization by *S. aureus* in the anterior nares (external part of the nostrils) (Iwase *et al.*, 2010). Thus, *S. epidermidis* can inhibit *S. aureus* colonization.

Pseudomonas auruginosa is another commensal, aerobic bacterial species. It is gram-negative and has flexible, non-stringent metabolic requirements, allowing it to occupy a variety of habitats. It is commonly considered a commensal bacterial strain, but can be an opportunistic pathogen in immunocompromised patients because their immune system is weakened, although disease related to *P. aeruginosa* is rare. On the other hand, *P. aeruginosa* can protect their human host from other pathogens. Some of its by-products, e.g. pseudomonic acid A (mupirocin) and PsVP-10, are so potent that they have been turned into commercial medications. *P. aeruginosa* is able to fully or partially inhibit not only *Staphylococci* and *Streptococci*, but also certain fungi, including many strains of *Candida*. Thus, *P. aeruginosa* can be considered a mutualistic bacterium (Cogen *et al.*, 2008).

The skin flora is not only composed of bacteria, although the great majority of skin microbiome research is focused on them, it also contains fungi. The most commonly found genus on the skin is *Malassezia* (Findley *et al.*, 2013), species *globosa*, *restricta* and *sympodialis*. Others that have been characterized include *Penicillium* (species *chrysogenum* and *lanosum*), *Aspergillus* (species *candidus, terreus* and *versicolor*), and a few *Alternaria*, *Candida*, *Chaetomium*, *Chrysosporium*, *Cladosporium*, *Mucor*, *Rhodotorula* and *Trichophyton* strains (Findley *et al.*, 2013).

3.2 Rosacea

3.2.1 Symptoms

Rosacea is a chronic skin condition that primarily affects the face. It starts with flushing, followed by telangiectatic vessels (spider veins) and persistent erythema (redness). In the progressive stage, inflammatory papules and pustules appear on the central facial area and hypertrophy (enlargement) of the sebaceous glands (secretion of oily or waxy matter) of the nose with rhynophyma (fibrosis). Patients may have increased sensitivity of the facial skin and may have dry, flaking facial dermatitis (Fimmel *et al.,* 2008). A classical staging of rosacea has been proposed by Plewig and Kligman (see *Table 4*). It can be described for long-term effects of rosacea patients (Jemec , 2010).

Table 4. Classical staging rosacea Plewig and Kligman. Source: modified from Plewig and Kligman, 2000.

Stage	Description
I	Prolonged erythema/cyanosis
	Teleangiectases
	Sensitive skin (stinging)
II	Appearance of inflammatory papules/pustules
	Oedematous papules
	Prominent pores
	More frequent attacks of inflammatory papules/pustules
	Involvement of larger areas of the face
III	Appearance of large inflammatory nodules (furunculoid elements)
	Tissue hyperplasia
	Oedema
	Phymata

Rosacea can also be divided into subtypes (see *Table 5*). The erythematotelangiectatic type (ETR) represents prolonged flushing caused by e.g. embarrassment, exercise or hot environments. It mostly occurs at the central part of the face but it may also involve the peripheral part of the face, ears, neck or the upper part of the chest. Patients with ETR can experience a stinging and burning sensation which can be quite severe. They have a lower threshold for irritation from topically applied substances which may exacerbate the stinging and burning. Papulopustular rosacea (PPR) patients often have a striking red central part on the facial skin. In addition, they have persistent or episodic inflammation characterized by small papules. The episodic inflammation may result in chronic edema. Phymatous rosacea portrays skin thickening and an irregular skin surface on e.g. nose, chin or forehead. This type of rosacea occurs mostly in men. Phymatous rosacea is not supposed to be vascular based or sun-induced like the other rosacea manifestations. Ocular rosacea represents mostly blepharitis (inflamed eyelids) and conjunctivitis (inflamed conjunctiva). Burning, stinging and light sensitivity occur (Crawford *et al.,* 2004). Sensory rosacea has not been recognized as a subtype by the National Rosacea Society Expert Committee (NRSEC). This type of rosacea can be cause by heat radiation or barrier disruption of the skin (Dahl, 2010).

Table 5. Rosacea subtypes and their clinical features. Source: Plewig and Kligman, 2000.

Subtype	Clinical features
Erythematotelangiectatic	Transient facial erythema (flushing and blushing)
	Persistent erythema (Erythema congestivum)
	Telangiectasias
	Edema
	Dermatitis
Papulopustular (i.e., inflammatory)	Persistent erythema (Erythema congestivum)
	Papules and pustules
	Plaques (cellulitis)
	Telangiectasias
	Demodex folliculitis
Phymatous	Localized skin tissue hypertrophy (inflamed or not inflamed)
	Sebaceous gland hyperplasia +/− fibrosis
	Hyperplastic phymas: rhinophyma (nose), chin (gnathophyma), forehead (metophyma), ears (otophyma), and/or eyelids (blepharophyma)
	Mucinous phymas
	Pseudorhinophyma
Ocular	Conjunctival hyperemia: Telangiectasia of conjunctiva and lid margin
	Corneal injury: Sensation of foreign body in the eye—Corneal complication (punctate keratitis, marginal keratitis, infiltrates, ulcers)
	Blepharitis
	Chalazion or hordeolum
Sensory	Pain (burning and stinging), pruritus, sensation of dryness, light sensitivity

3.2.2 Quality of life patients

Rosacea has a great psychological impact. Nicholson *et al.* (2007) tried to develop a validated, reliable rosacea-specific instrument to indicate the quality of life of a rosacea patient. In *Table 6* an overview is presented of a few questions that were asked to rosacea patients (Nicholson *et al.,* 2007). The scores range from 1 (nothing) to 4 (severe) and the percentage of patients that indicate the specific (highest) score are given. Patients avoid certain environments, need medication for side effects, avoid certain food or drinks and some are bothered with their eyes. This means they may not be able to live life to the fullest and cannot do everything they want to. Furthermore, rosacea patients are self-conscious, worried, frustrated and annoyed. Some patients are even depressed like in other skin diseases (Chosidow and Cribier, 2011). Therefore it is important that these patients receive treatment to reduce the symptoms found in rosacea.

Table 6. Rosacea patients' impact scores. Source: Nicholson *et al.*, 2007.

Question	Percentage	Median Score
Appearance of my skin	16.4	3.0
Self-conscious	36.1	2.0
Cover up rosacea	39.3	3.0
Bothered by persistence	34.4	3.0
Avoid food or drinks	52.5	1.0
Feels bumpy	37.7	3.0
Flushes	6.6	4.0
Skin is irritated easily	27.9	3.0
Eyes bother me	52.5	1.0
Think about my rosacea	31.1	2.0
Avoid certain environments	57.4	1.0
Serious	59	1.0
Burns or stings	54.1	1.0
Worry about scars	59	1.0
May get worse	29.5	3.0
Medication side effects	57.4	1.0
Irritated	37.7	2.0
Embarrassed	45.9	2.0
Frustrated	41	2.0
Sensitive skin	21.3	3.0
Annoyed	34.4	3.0

3.2.3 Risk factors

The cause of rosacea is still unknown but several factors are suspected to contribute to disease. Familial cases of rosacea have been reported which indicates a genetic background. Recently, also sun damage, mites, bacteria, stress and other factors are suspected to contribute to disease (McAleer and Powell, 2010).

Family history

Patients with rosacea have a significantly greater chance of having other family members affected by rosacea compared to non-rosacea controls. This is caused by either genetic factors or common environmental factors. One underlying genetic mechanism could be a polymorphism (mutation) in glutathione S-transferases (GSTs). These GSTs encode enzymes that catalyze detoxification of UV induced oxidative stress like ROS. If these enzymes do not work correctly due to a polymorphism, less ROS detoxification occurs. This may explain the correlation of UV (ultraviolet) exposure and rosacea on a genetic basis. GSTM1 and GSTT1 are polymorphisms that are associated with a higher risk of rosacea. However, also environmental factors play a role in the occurrence of rosacea by the observation that rosacea only affected one of a pair of monozygotic (identical) twins (Tan and Berg, 2013).

Age, gender and skin type

Rosacea is the most prevalent in adults over the age of thirty. However, this may be biased because studies have only been conducted in adults. The distribution of the disease between genders is almost equal with slightly more prevalence in females. Most of the time women have erythematotelangiectatic, papulopustular or ocular rosacea while phymatous rosacea mostly occurs in men. Rosacea is more prevalent in lighter skin (Tan and Berg, 2013). Caucasians with skin type I

and II have an increased sensitivity and an abnormal barrier function of the skin which facilitates a high population of *Demodex* mites (Lacey and Powell, 2010).

Sun damage

Sun damage is an inflammatory process that can cause telangiectasia (spider veins) and erythema. Therefore, sun-exposed sites like the face, neck and upper part of the chest are frequently affected (Crawford *et al.,* 2004). UV radiation is a factor that contributes to the development of inflammatory lesions in rosacea. However, some studies failed to show acute effects of UV radiation on the skin. UVB radiation down regulates thrombospondin-1 (inhibits angiogenesis) and up regulates the vascular endothelial growth factor (promotes angiogenesis) which results in angiogenesis (formation of new blood vessels). These new blood vessels facilitate the movement of inflammatory cells, like neutrophils and macrophages, into the dermal tissue which causes damage of the derma. Vascular endothelial growth factor was found in infiltrating cells and epithelial cells of rosacea patients. UV light targets Langerhans cells (LC) and keratinocytes. UV radiation inhibits the antigen presentation of LC and thereby inhibits the capacity to stimulate T-cells. When keratinocytes are targeted by UV light they produce and release multiple immunosuppressive factors like IL-10 (Fimmel *et al.,* 2010). The adaptive immune response is inhibited by UV light. However, UV light does result in an innate immune response and angiogenesis in the skin which causes inflammation.

Staphylococcus epidermidis

Staphylococcus epidermidis is a bacterium that normally inhabits the human skin and is an opportunistic pathogen (Otto, 2009). This means it only causes disease when the host is weakened. Often, the papules and pustules of rosacea patients disappear when they receive an antibiotics treatment. This indicates that bacteria cause the inflammation and these papules and pustules. The temperature of the facial skin of rosacea patients is increased compared to healthy individuals (Dahl *et al.,* 2004). The higher temperature is a result of the consistent erythema and higher blood flow. Importantly, bacteria behave differently at higher temperatures and growth rates are higher. Bacteria, such as *S. epidermidis*, produce and secrete more proteins at a higher temperature. These proteins can cause activation of PRRs and cause inflammation. In addition, the *staphylococci* found on the skin of rosacea patients were consistently β-haemolytic (have the ability to rupture red blood cells) whereas those of the control subjects were non-haemolytic (Dahl *et al.,* 2004). However, the growth rate and density of *S. epidermidis* was the same in both patients and control subjects. One protein which is upregulated at higher temperatures is a lipase which indicates that *S. epidermidis* is more virulent or secretes more virulent factors in skin of rosacea patients. It is not certain whether *S. epidermidis* is linked to rosacea but it is likely that the higher facial temperature affects the microflora on the skin. It is found that the microbial composition of the skin follicles and the skin surface is different compared to control patients (Dahl *et al.,* 2004).

Demodex folliculorum

In humans only two species of mites have been identified: *Demodex folliculorum* and *D. brevis. D. folliculorum* mites are found in the infundibular (cavity) portion of hair follicles, while *D. brevis* burrows deeper into the sebaceous glands and ducts. *D. folliculorum* is a mite that is present in the skin of adult humans. The role of mites in rosacea is controversial. Some studies have shown that there is a higher number of mites in rosacea patients compared to controls patients (Erbagči and Özgöztaşi, 1998). Sebum, made by sebaceous glands, is proposed to be the main food source of the mite. There are specialized piercing mouth parts present in the mouth of the mite. This could indicate

feeding on epithelial cells and disruption of the cell walls. *D. folliculorum* has a life cycle of around 14 days. Mites are probably transmitted through contact of adults with children and are mostly found in areas rich in sebaceous glands. *D. folliculorum* is regarded as a commensal organism and in most humans they do not initiate inflammation. However, their mode of action has not been investigated yet (Lacey and Powell, 2010).

The increase in *D. folliculorum* numbers in the spring and summer is parallel to the increase of rosacea in these months. There are also studies showing an increase in *Demodex* mites in patients with a supressed immune system due to chemotherapy, HIV infection or chronic dialysis. Mites may mechanically block hair follicles and sebaceous ducts by either increased numbers or hyper keratinization and hyperplasia of epithelial cells. This could promote bacterial overgrowth of e.g. *S. epidermidis* and induce inflammatory lesions. Papulopustular rosacea patients have an increased skin pH (more alkaline), reduced skin hydration levels and an altered fatty acid profile (Raghallaigh and Powell, 2009; as cited in Lacey and Powell, 2010). This could be caused by *D. folliculorum,* which feeds on sebum. When *D. folliculorum* numbers increase they may rupture epithelial cells with their specialized mouth parts. The mites or their related antigens will then penetrate the dermis or epithelial surface and initiate an immune response (Lacey and Powell, 2010). It is not clear which kind of immune reaction takes place. Some studies demonstrate a CD4+ Th-cell increase in the derma (Georgala *et al.,* 2001) and others indicate a humoral (antibody) response from B-cells. Because the life cycle of a mite is short, large numbers of dying and dead mites are present. These could release their waste products into a damaged follicular canal, which promotes inflammation. In addition, inflammation can occur due to their chitinous exoskeleton (Lacey and Powell, 2010). Some similar inflammatory reactions are also seen in asthma patients against house dust mites (Nathan *et al.,* 2009). Furthermore, the enzymes released by the mites, like lipases or proteases, can cause an immune reaction. Some *Demodex* mites are also covered by alpha-1-antitrypsin and alpha-1-antichymotrypsin, which are serum protease inhibitors and protect the host. Mites have symbiotic endobacteria which, when released from dead mites into follicles, can cause inflammation (Lacey and Powell, 2010). In one study, it was found that *Bacillus oleronius*, isolated from *Demodex* mites, induces mononuclear cells (macrophages and lymphocytes) in rosacea patients (Lacey *et al.,* 2007). Moreover, various bacteria adhere to the chitinous skeleton of mites which could also initiate inflammation. The different consequences of the presence of *D. folliculorum* on the skin are summarized in *Table 7*.

Helicobacter pylori

Helicobacter pylori is a gram negative bacterium that colonizes the gastric mucosa and when cytotoxic substances are released it induces inflammation. The presence of the bacterium is associated with gastritis, ulcers and gastric cancers. *H. pylori* produces toxins and enzymes that cause mucosal inflammation of the stomach and duodenum. Patients with a *H. pylori* infection in the gastric mucosa have elevated levels of TNFα, IL-1b, and IL-8 (Tüzün *et al.,* 2010). A first association with rosacea was made when the rosacea skin symptoms disappeared when patients were treated for their gastritis with metronidazole (Utaş *et al.,* 1999). However, the disappearance might be explained by the antioxidant effects of metronidazole (Abram *et al.,* 2010). In one study a significantly higher number of *H. pylori* were present in rosacea patients compared to controls. Furthermore, in 62% of rosacea patients with *H. pylori* gastritis was confirmed (Argenziano *et al.,* 2003). However, the effect of *H. pylori* on rosacea is unknown. It is thought that *H. pylori* causes the

production of ROS, pro-inflammatory cytokines and nitric oxide. Nitric oxide is a vasodilator and causes an increased blood flow and therefore rosacea. In patients with gastritis specific IgA and IgG antibodies were found against *H. pylori*. Rosacea patients with gastritis also had anti-CagA antibodies (Argenziano *et al.,* 2003). CagA antibodies stimulate the production of the inflammatory cytokines IL-8 and macrophage inflammatory protein 1α and 1β (MIP-1α and 1β) by the epithelium. These cytokines attract T-lymphocytes, neutrophils, macrophages, eosinophils, basophils and neutrophils. It is possible that the toxins produced by *H. pylori* can enter the circulation and end up in the endothelium and cause inflammation at other sites in the body. Furthermore, IL-8, and interleukins produced due to the immune response against *H. pylori*, TNFα and IFNγ also enter the circulation and could also be a cause of rosacea (Tüzün *et al.,* 2010).

Table 7. Possible actions and consequences of mites on the skin. Source: Lacey and Powell, 2010.

Possible action		Potential consequences
1	Altered cutaneous microenvironment	The cutaneous microenvironment of rosacea patients may prove conducive for the proliferation of *Demodex*. At a critical number the host immune response may be triggered to reduce numbers to an acceptable level
2	Mechanical blockage of follicles	The number of mites could obstruct normal sebum flow and cause stagnation. Resultant host immune stimulation or bacterial overgrowth of *P. acnes* or other bacteria may induce inflammatory lesions
3	Alteration of sebum composition	*Demodex* mites may alter sebum composition; by selectively ingesting particular constituents, by changing the pH of sebum or by facilitating normal flora overgrowth
4	Alter local immune reactivity	*Demodex* may be able to downregulate the host's immune response, only becoming an opportunistic pathogen with the host's immune system is altered. The aberrant innate immune response in rosacea patients could allow the proliferation of *Demodex* to a critical number where the adaptive immune response is initiated and cutaneous inflammation occurs
5	Damage to follicular epithelium by mites	*Demodex* mites may rupture follicular epithelial lining cells by way of their specialised mouth pieces, with subsequent inflammatory reaction. Rupture of follicles with granulomatous reaction could occur in severe cases
6	Release of waste products by mites	Large numbers of dying mites could release crystalline waste products into follicular canal initiating inflammation
7	Release of endogenous enzymes	*Demodex* mites have been shown to possess enzymes such as lipase to facilitate digestion of lipids. Release of these may facilitate inflammation. *Demodex* may have other proteases that may dysregulate the endogenous protease/protease inhibitor balance in the skin
8	Endobacterial release from degenerating mites	Mites may have symbiotic endobacteria which cause immune reaction in host when released from dead mites
9	Surface bacterial transportation	Mites may transport bacteria on their outer surface from other follicles initiating inflammation

Psychological stress

Psychological stress, such as anxiety or depression, alone or in combination with processed food without fibres, can lead to increased intestinal permeability. Systemic inflammation and substance P (SP) levels are increased while insulin sensitivity is decreased (Bowe and Logan, 2011). Substance P is a peptide released by sensory nerve cells that causes the release of inflammatory substances (see also the Molecular Mechanisms part about neuropeptides) (Powell *et al.,* 1993). In patients who are already sensitive to developing rosacea due to their genes, this cascade is believed to exacerbate the condition (Bowe and Logan, 2011).

Oxidative stress

When a cell is under stress, ROS are produced. The involvement of ROS in rosacea is demonstrated by the decrease of symptoms when treated with compounds that have an antioxidant effect e.g. tetracycline, azelaic acid and azithromycin (Yamasaki and Gallo, 2009). A study also confirmed a higher ROS activity in skin lesions of rosacea patients compared to healthy skin (Peus *et al.,* 1999). ROS can cause oxidative damage to nucleic acids, sugars, proteins and lipids which can eventually lead to protein degradation and apoptosis. The skin has a mechanism that produces anti-oxidants for protection against ROS. In the skin, more damage is caused by ROS compared to other body parts because the skin is exposed to two important factors, UV radiation and oxygen, are present in the skin. UV light causes depletion of anti-oxidants and SOD (superoxide dismutases; anti-oxidant enzymes). The results are DNA damage, activation of the neuro-endocrine system and increased production of pro-inflammatory molecules. This leads to more neutrophils and phagocytes in the skin (Narayanan *et al.,* 2007). Severe rosacea can be caused by a defective antioxidant system in the skin (Öztas *et al.,* 2003). The higher levels of ROS in the skin of rosacea patients can be a result from overproduction or inadequate removal of ROS or both. The suspected mechanism in the skin of rosacea patients is that ROS damages the skin and causes inflammation (Narayanan *et al.,* 2007).

Problems in the small intestine

Rosacea patients have a higher frequency of bacterial overgrowth in the small intestine (SIBO) compared to healthy individuals (Whitehead, 2009). When the affected patients are treated for SIBO, the rosacea decreases (Parodi *et al.,* 2008 as cited in Whitehead, 2009). SIBO is associated with several neurological symptoms and diseases which decrease when treated for SIBO (Pimentel *et al.,* 2001; Finegold *et al.,* 2002). Therefore, a treatment for SIBO could reduce vasodilatation and other neuropeptide-involved rosacea symptoms.

Intestinal alkaline phosphatase (IAP) is an enzyme expressed on surface epithelial cells in the gut. They detoxify LPS or endotoxins from gram-negative bacteria. This inhibits inflammation, since the immune system does not get to recognize these MAMPs. If these IAPs are not produced, pro-inflammatory cytokines, like TNFα, are produced in response to LPS (Whitehead, 2009). TNFα can induce a local but also a systemic immune response (Bates *et al.,* 2007). One third of rosacea patients has abnormal small intestinal tissue (Watson *et al.,* 1965). It could be that they have less IAPs compared to healthy individuals. In addition, when rosacea patients received oral administration of IAPs, their symptoms reduced (Whitehead, 2009).

The optimal pH of IAP is around 9.5. However, it also works efficiently at a pH of 7.5 in the small intestine using LPS as a substrate (Poelstra *et al.,* 1997). Since the western diet is more acidic, the IAPs can work less efficiently and therefore can cause rosacea. An alkaline diet might improve the symptoms of rosacea (Whitehead, 2009). The IAPs are also influenced by phytates (Bitar and Reinhold, 1972), which are found in grains and legumes, and phenylalanine, an amino acid part of the artificial sweetener aspartame (Whitehead, 2009). Wheat bran worsens rosacea symptoms and avoiding it results in improvement of symptoms (Kendall, 2004). SCFAs like butyrate and dietary fish oil increase IAP activity. Furthermore, dietary zinc and vitamin A activate IAPs (Whitehead, 2009).

3.2.4 Molecular mechanisms

The mechanisms that cause rosacea are poorly understood. Chronic inflammation and vascular changes are believed to be factors underlying this disease.

Increased levels of cathelicidin

The positive correlation between specific bacteria or mites and rosacea indicate that the external environment affects rosacea. These environmental factors are recognized by specific intrinsic factors of the host. The clinical manifestations of rosacea patients can be explained by an inappropriate response of the innate immune system. In innate immunity, pattern recognition receptors (PRR) respond to specific global patterns on microbes, UV light and trauma. This leads to an increase of chemokines, cytokines and antimicrobial molecules.

One of these antimicrobial molecules is cathelicidin. This molecule is hardly detectable in normal skin but, when the skin is wounded, production of cathelicidin is induced. Cathelicidin has different forms, these different forms can be both vasoactive and pro-inflammatory (Yamasaki and Gallo, 2011). Cathelicidins are produced by keratinocytes and neutrophils. Cathelicidin levels in rosacea patients are increased and the forms in present rosacea patients affect both vasoactive and pro-inflammatory properties. These forms do this via the promotion and regulation of leukocyte chemotaxis and angiogenesis. This means the movement of leukocytes to certain tissues and the formation of new blood vessels is affected.

The cathelicidin LL-37 is the most commonly found cathelicidin form in rosacea patients and is typically present in neutrophils in the injured or infected skin (Yamasaki *et al.,* 2007). Cathelicidin LL-37 promotes angiogenesis via epidermal growth factor which induces vascular endothelial growth factor in keratinocytes.

The increased level of cathelicidin is due to an increase in the amount of TLR2 receptors present on the skin of rosacea patients. The TLR2 receptor is more expressed on the cell surface in rosacea patients compared to healthy individuals. The increased amount of TLR2 receptors leads to an increase in activation by specific stimulators. This sets off a cascade of reactions which results in an increased level of cathelicidins (Yamasaki *et al.,* 2010).

Patients with rosacea do not only have an increased cathelicidin level but also a higher level of serine protease activity. This protease cleaves cathelicidin into the active form (Yamasaki *et al.,* 2007). One of the serine proteases which is upregulated is Kallikrein 5 (KLK5). (Yamasaki *et al.,* 2007). Matrix metalloproteinases (MMPs) turn the inactive KLK preproenzymes into active KLKs (Kanada *et al.,* 2012). This KLK5 processes a precursor of cathelicidin into cathelicidin (Yamasaki and Gallo, 2011).

TLR2 activation activates vitamin D into active vitamin D. Active vitamin D increases cathelicidin production by keratinocytes. This is done by amplifying the pathway from TLR2 activation to cathelicidin production (Schauber *et al.,* 2006).

The endobacterium *B. oleronius,* which resides inside *D. folliculorum,* secretes heat-shock proteins and a lipoprotein (Lacey *et al.,* 2007). It is known that these proteins stimulate TLR2 (Costa *et al.,* 2002) and therefore can cause inflammation.

Neuropeptides

Stress, UV light or microbes can stimulate the release of specific neuropeptides (see *Figure 15*). This can contribute to the symptoms like flushing and erythema. Dilated vessels cause an increased blood flow and leak fluid and pro-inflammatory molecules. The results are pustules, papules and flushing. Some patients also have symptoms like itching and burning. This suggests that the cutaneous

neurovascular system plays a role. Several cutaneous neuropeptides have been studied (see *Table 8*) that are produced in dorsal root ganglia (part of the spinal nerve cells) (Slominski and Wortsman, 2000).

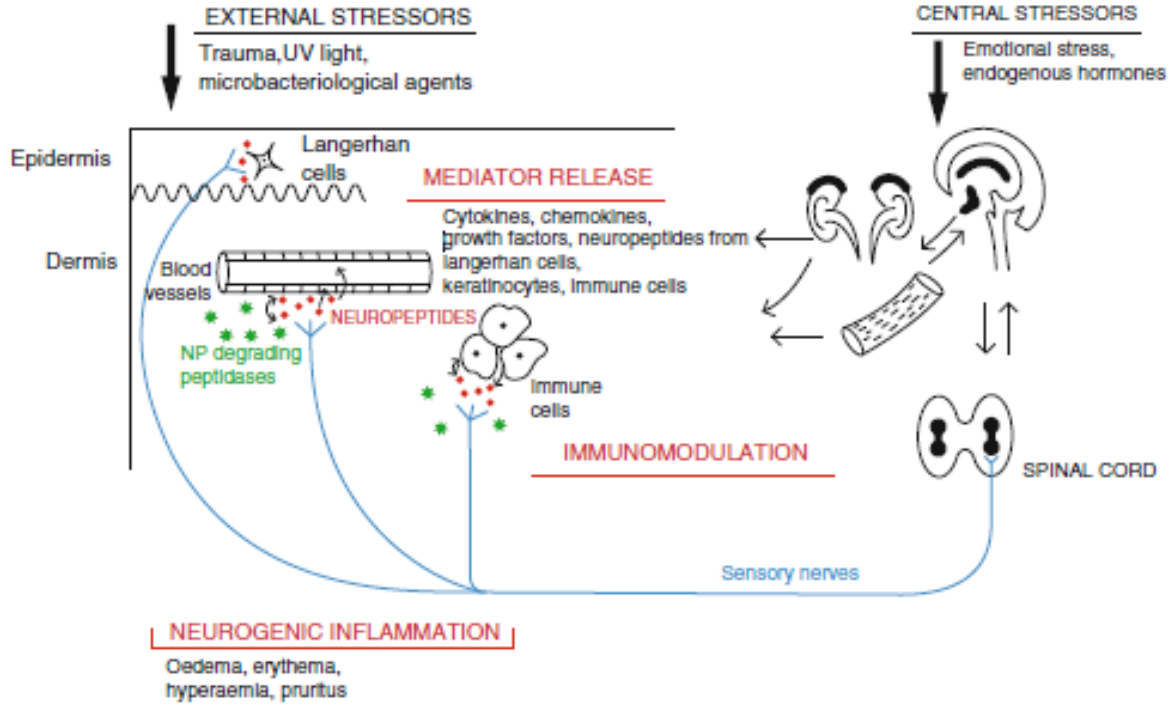


Figure 15. Inflammation in the skin caused by external or internal factors. Soucre: Parnham, 2011.

Table 8. Overview of cutaneous neuropeptides potentially involved in rosacea. Source: McAleer and Powell, 2010.

Neuropeptide	Receptors	Main sources in the skin	Effects	Characterisation in rosacea
SP	Tachykinin receptors	– Sensory nerves	– Erythema, oedema and itch – Histamine release from mast cells – Vasodilatation – T cell and macrophage activation, neutrophil chemotaxis – KC and fibroblast proliferation – ↑ Cell adhesion molecules – ↑ IL, TNF-α, leukotrine B_4, prostaglandin – Sebaceous gland regulation	– Increased serum SP in rosacea [11] – Increase in SP immunoreactive nerves in lesional skin of rosacea patients [12] – Following pulsed dye laser rosacea patients had reduced stinging and a reduction in neurons immunoreactive to SP [4]
VIP	VPAC receptors	– Sensory nerves – Merkel cells	– Pro and anti-inflammatory effects – Sweat secretion – Histamine release from mast cells – Induces NO synthesis – Vasodilation and blood vessel function – Keratinocyte migration and proliferation – Involved in IL, chemokine, TNF-α release	– Denser distribution of VIP receptor positive cells in the endothelium and perivascular large cells of patients with rhinophyma compared with the control group [13]
CGRP	CGRP receptors	– Sensory nerves	– Vasodilatation – Mast cell degranulation – Histaimine & TNF-α release – Pro and anti-inflammatory effects – Release of nitric oxide – Regulation of keratinocyte and endothelial cell proliferation	– No significant change in CGRP immunoreactive neurons after pulsed dye laser treatment [4]
SST	SST receptors	– Sensory nerves	– Histamine release from mast cells – Regulation of T and B cell proliferation – Antiproliferative actions	– Case reports of patients with papulopustular rosacea responding to octreotide (SST analogue) [14]
NKA	Tachykinin receptors	– Sensory nerves	– Histamine release from mast cells – ↑ Keratinocyte nerve Growth factor expression	– Post prandial flushing was observed in 48 % of rosacea patients compared to 0 % of controls. Post prandial NKA levels were increased on 7 % of rosacea patients and 26 % of controls. No association between flushing and neuropeptides or rosacea and neuropeptides was demonstrated [15]
CRH	CRH receptors	– Keratincytes – Pilosebaceous unit – Melanocytes	– Proinflammatory – Mast cell degranulation; release of histamine, TNF-α, cytokines and VEGF from mast cells – Fibroblast proliferation, anti-proliferative in keratinocytes – Stimulates steroid production	– Epithelial cells respond to environmental stress by increased production of CRH and this has direct effects on vessel wall function [16]

Substance P (SP) is a peptide released by sensory nerve cells that causes the release of inflammatory substances. This results in release of TNFα and histamine. SP also upregulates cytokines production, releases chemokines and recruits neutrophils and eosinophils. In addition, the neurons in rosacea patients were more reactive towards SP. Rosacea patients have an increased level of SP in their skin compared to healthy individuals (Powell *et al.,* 1993).

Vasoactive Intestinal Peptide (VIP) is a peptide which is present in gastrointestinal and neural tissue. The peptide regulates vasodilatation by inducing the synthesis of nitric oxide (McAleer and Powell, 2010). In phymatous rosacea patients there is more VIP present in the skin compared to healthy individuals (Wollina, 1996).

Calcitonin gene related peptide (CGRP) is a neuropeptide which is expressed in the central nervous system, neurons near blood vessels and in nerves ending in the skin. Furthermore, it is found in smooth muscle and blood vessels. CGRP has a mostly anti-inflammatory effect but can be pro-inflammatory in the early stages of inflammation. CGRP has an effect on small and large blood vessels, causes permeability of the vessels and acts on endothelial cells. The endothelial cells react with the release of nitric oxide. This causes vasodilatation and flushing, the symptoms seen in rosacea patients. Some rosacea patients have elevated CGRP levels compared to healthy individuals.

Somatostatin (SST) is found in Merkel cells and is associated with e.g. LC, keratinocytes and neurons (McAleer and Powell, 2010). SST causes inflammation in rosacea patients.

Corticotropin releasing hormone (CRH) is a peptide that is released in response to chronic stress, UV light and cytokines. It has both pro- and anti-inflammatory effects. CRH degranulates mast cells, which contain histamine, and thereby increase vascular permeability and induce keratinocyte differentiation (McAleer and Powell, 2010). UVB radiation increases CRH in keratinocytes and fibroblasts while it decreases CRH in endothelial cells (Fimmel *et al.,* 2005). In rosacea patients, CRH can contribute to vasodilatation and flushing.

3.2.5 Interactions between the possible risk factors and molecular mechanisms

In rosacea TLR2 receptors are more expressed on the cell surface of amongst others skin cells, compared to healthy individuals (Yamasaki *et al.,* 2010). TLR2 receptors recognize many different MAMPs like lipoproteins, lipoteichoic acid, peptidoglycans, phenol-soluble modulin, zymosan and LPS forms. These LPS forms are present on non-enterobacteria like *H. pylori* (Takeda and Akira, 2005). In rosacea patients, the facial skin temperature is higher compared to controls. *S. epidermidis* produces and secretes more proteins at a higher temperature (Dahl *et al.,* 2004). One factor that is secreted is phenol-soluble modulin. This factor acts as a ligand for TLR2 (Hajjar *et al.,* 2001). When TLR2 receptors get activated, a signalling cascade leads to the activation of NFκB, JNK and p38 (see *Figure 16*). JNK and P38 are both involved in inflammation. NFκB is a transcription factor that is essential for the transcription of several immune system related genes. These include adhesion molecules that regulate the recruitment of immune cells to the site of infection and pro-inflammatory cytokines like IL1 and TNFα (Medzhitov, 2001). NFκB also regulates the transcription of iNOS (Zhang and Ghosh, 2001). iNOS (inducible nitric oxide synthesis) is an enzyme that produces nitric oxide in response to cytokines or bacterial products (Aktan, 2004).

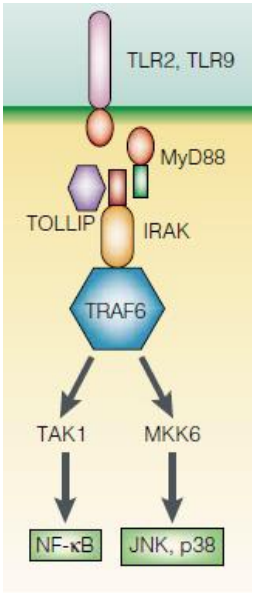


Figure 16: Signalling pathway of TLR2 (Medzhitov, 2001).

Neutrophils are immune cells with respiratory bursts that contain ROS. These ROS act against pathogens but can induce damage to the tissue of the host (Nathan, 2006). In rosacea the ROS levels are increased (Peus *et al.,* 1999). Neutrophils have TLR2 receptors that, when activated, initiate the production of cathelicidins, nitric oxide and ROS (Nathan, 2006). In rosacea patients these three substances have elevated levels compared to healthy individuals (Peus *et al.,* 1999; Yamasaki *et al.,* 2007; McAleer and Powell, 2010). The cathelicidin LL37 is important in protection against skin infections caused by *Streptococcus* bacteria (Nizet *et al.,* 2001).

S. epidermidis forms a biofilm which is a multicellular agglomeration. A biofilm has a characteristic physiology and architecture that confers resistance to many antibiotics and host defence mechanisms (Otto, 2009). In this way, *S. epidermidis* is resistant to cathelicidins like LL37. Rosacea patients have more active *S. epidermidis* on their skin (Dahl *et al.,* 2004) and therefore more TLR2 receptors are activated (Hajjar *et al.,* 2001). If in some way *S. epidermidis* is inhibited or if the bacterium has competition, the symptoms of rosacea might be less severe. Heparin, an anti-coagulant produced by mast cells and basophils, interferes with *S. epidermidis* anchorage to the surface (Arciola *et al.,* 2003).

3.2.6. Treatments

Currently, there is no treatment available to cure rosacea. The treatments developed are to control the symptoms. There are topical and oral treatments possible. The treatments used are summarized in *Tables 9* and *10*.

Topical

In *Table 9* there is a short summary of topical treatments currently used for rosacea. The Food and Drug Administration (FDA) approved four topical treatments for rosacea including metronidazole and azelaic acid. Metronidazole has anti-oxidant, anti-inflammatory and antimicrobial effects (Narayanan *et al.,* 2007). It is used to treat erythema and inflammatory lesions (Veldman *et al.,* 2014). The mechanism of action is believed to be a decrease of ROS in neutrophils (Narayanan *et al.,* 2007).

Azelaic acid also has anti-oxidant, anti-inflammatory and antimicrobial effects. It is used to treat papules and pustules of mild and moderate rosacea (Veldman *et al.,* 2014). The mechanism of action may be through anti-inflammatory and anti-keratinization effects (Mayer-da-Silva *et al.,* 1988). Furthermore, azelaic acid downregulates KLK5 and therefore downregulates cathelicidins (Coda *et al.,* 2013).

Laser and light therapies are currently used as a treatment for rosacea. Telangiectasia, remodelling of the connective tissue and strengthening of the skin barrier are the targets of laser and light therapies. There are a few ways in which lasers could have an effect on the skin: cell proliferation and cytokine, growth factor and heat shock protein activation by endothelial disruption. Two types of therapies are used for rosacea: vascular lasers and intense pulsed light. Vascular lasers emit light that is absorbed by oxyhemoglobin and results in vessel destruction. No collateral damage is found because of a principle called 'selective photothermolysis'. With shorter wavelengths the superficial red vessels, which cause persistent erythema, are targeted (Pelle *et al.,* 2004). Pulsed dye laser reduced erythema and tangiectasia in patients with rosacea. In addition, patients who used antibiotics could reduce the dosage when treated with pulsed dye laser (Lowe *et al.,* 1991). However, hyperpigmentation occurs with pulsed dye laser. Intense pulsed light is a multichromatic light with multiple wavelengths. This goes deeper into the skin than light from a vascular laser and has multiple targets like melanin and hemoglobin (Pelle *et al.,* 2004). It is used for the treatment of vascular lesions, unwanted hair and photoaging. It may induce facial rejuvenation by inducing cytokines and growth factors which contribute to tissue remodelling (Weiss *et al.,* 2002). Side effects like edema, hypopigmentation and bruising occurs (Angermeier, 1999). Since it improves vascular lesions and contributes to tissue remoddeling, intense pulsed light laser improves the symptoms seen in rosacea.

Some possible topical treatments for rosacea are still in development e.g. cyclosporin A, nicotinamide. Cyclosporin A could be an effective treatment for ocular rosacea. It has anti-inflammatory properties by inhibiting NFkB activation by lipopolysaccharides (innate immunity) and by inhibiting proteasome (for degradation proteins) activity (Wollina, 2014).

In rosacea hydration levels of the skin are lower compared to healthy skin. The dry skin in rosacea patients indicate a disruption of the skin barrier and the associated fatty acids (Ní Raghallaigh and Powell, 2014). A treatment that could reduce symptoms in rosacea by improvement of the barrier function of the skin is nicotinamide. Nicotinamide is a B vitamin (vitamin B3) that increases the synthesis of ceramides and other lipids that are present in the skin. Ceramides are lipids that contribute to the skin barrier. In addition, topical treatment also increases free fatty acids and cholesterol in the skin (Tanno *et al.,* 2000). Nicotinamide is already used for a few skin conditions and could also be used for other inflammatory skin diseases. Furthermore, there is evidence it works against aging of the skin and it may be a potential chemo preventive agent against skin cancer (Chen and Damian, 2014).

A topical minocycline treatment could complement an oral treatment (see below). The dry skin in rosacea patients indicate a disruption of the skin barrier and the associated fatty acids. The skin of some rosacea patients is more alkaline than healthy skin (Ní Raghallaigh and Powell, 2014). Some enzymes of the skin function best in a more acidic environment (Hachem *et al.,* 2003). In rosacea patients these enzymes do not have an optimal environment. Furthermore, protease enzymes from microbes work best in a more alkaline environment (Hachem *et al.,* 2005). These two result in a

disrupted skin barrier in rosacea patients. A topical treatment with minocycline reduces the pH so the enzymes of the skin work better and the enzymes of the microbes work less. Therefore a topical use of complements an oral use of minocycline (Ní Raghallaigh and Powell, 2014).

Oral

In *Table 10* there is a short summary of oral treatments currently used for rosacea. Tetracyclines are a class of antibiotics most commonly used in rosacea treatment. It is believed they have an effect on angiogenesis, cell proliferation and inflammation. They also modulate the immune system (Sloan and Scheinfeld, 2008). The reduction of inflammation may be because of the reduction of pro-inflammatory cytokines (Weinberg, 2005). Doxycycline and minocycline are from the antibiotics class of tetracyclines. Doxycycline is the most used antibiotic for rosacea, is anti-inflammatory and reduces lesions in rosacea patients (Veldman *et al.,* 2014). Matrix metalloproteinases (MMPs) are zinc enzymes that are responsible for degradation of the extracellular matrix components like collagen, which is a structural protein in connective tissue in the skin (Woessner, 1991). One mechanism by which doxycycline may reduce symptoms in rosacea patients is inhibition of KLK activity and the LL-37 peptide by inhibiting the MMPs in keratinocytes (Kanada *et al.,* 2012). New blood vessels (angiogenesis) have to be formed out of existing ones. This happens by removal and remodelling of the vascular membrane by MMPs (Rundhaug, 2005). Therefore, inhibiting MMPs also results in less angiogenesis and less flushing in rosacea patients.

Several oral treatments for rosacea are still in development. One treatment that could possibly reduce rosacea symptoms is minocycline. Minocycline treatment reduces the erythema, papules and pustules of PPR patients. In addition, the skin hydration levels improved significantly after topical treatment with minocycline (Ní Raghallaigh and Powell, 2014) (see topical treatments).

Table 9. Topical treatments in different types of rosacea. Source: Dahl, 2010.

Subtype	Treatment
Erythematotelangiectatic	
Erythema	Metronidazole Azelaic acid Cover-ups
Erythema congestivum (i.e., persisting erythema)	Metronidazole Azelaic acid Cover-ups Intense pulsed light
Flushing or blushing	Cool compresses
Edema	Cool compresses Control flushing
Telangiectases	Intense pulsed light (for small vessels) Vascular laser
Papulopustular	
Papules and pustules	Metronidazole Azelaic acid Sulfacetamide or sulfur Benzoyl peroxide Benzoyl peroxide or clindamycin Clindamycin Tretinoin
Cellulitis	Intralesional steroids
Demodex folliculitis	Sulfacetamide Sulfur Permethrin
Dermatitis	Metronidazole Ketoconazole Sulfacetamide or sulfur
Phymatous	
Hyperplastic phymas	Ablative laser Electrosurgery
Mucinous phymas	Metronidazole Topical corticosteroids
Pseudorhinophyma	Remove heavy glasses
Ocular	Eyelid hygiene (i.e., use baby soap wash) Topical sulfacetamide
Sensory	
Pain (i.e., burning, stinging)	Wash face (i.e., use cleanser and water) Moisturize (i.e., use bland emollients) Sulfur
Itching	Calcineuron inhibitors Hydrocortisone

Table 10. Oral (systemic) treatments in different types of rosacea. Source: Dahl, 2010.

Subtype	Treatment
Erythematotelangiectatic	
Erythema	Tetracycline Doxycycline
Erythema congestivum (persisting erythema)	Tetracycline Doxycycline
Flushing or blushing	β-Adrenergic blocking agents α-Adrenergic agonists Anticholinergics
Edema	Isotretinoin (for solid facial edema)
Telangiectases	None available
Dermatitis	Tetracycline
Papulopustular	
Papules and pustules	Tetracyclines (e.g., tetracycline, oxytetracycline, doxycycline, or minocycline) Macrolide antibiotics (e.g., erythromycin, azithromycin, clarithromycin) Ampicillin Trimethoprim-sulfamethoxazole Metronidazole Isotretinoin
Cellulitis	Doxycycline Intralesional or systemic corticosteroids
Demodex folliculitis	Ivermectin
Phymatous	
Hyperplastic phymas	Isotretinoin (temporary)
Mucinous phymas	Doxycycline
Ocular	Doxycycline Tetracycline
Sensory	
Pain (e.g., burning)	Nonsteroidal anti-inflammatory agents Doxepin
Itching	Hydroxyzine

3.2.7 Summary rosacea

- Rosacea is a chronic skin condition mainly affecting the face
- Symptoms include flushing, redness and sometimes papules and pustules
- Risk factors include family history, age, gender, skin type, exposure to UV radiation, psychological stress and oxidative stress
- The presence of *Demodex* mites and *Helicobacter pylori* may also contribute
- *Staphylococcus epidermidis* is more active in rosacea due to increased skin temperature
- Mechanisms underlying rosacea are poorly understood
- Potential underlying mechanisms include increased cathelicidin (e.g. LL-37) levels and increased release of specific neuropeptides (e.g. substance P)
- No cure, but symptom management using topical and oral treatments
- Often treated with antibiotics

3.3 Acne

Acne (vulgaris) is a common disease that mainly affects teenagers. About 80% of the people acquire acne in their adolescence (Cunliffe and Gollnick, 2001). People can experience acne symptoms for decades; nearly 50% of these people suffer from acne in their 20s (Al Robaee, 2005; Galobardes *et al.,* 2005). By the age of 40, 1% of men and 5% of women still have acne lesions (Goulden, 1999).

3.3.1 Symptoms

The typical clinic appearance of acne can be non-inflammatory or inflammatory. Non-inflammatory acne lesions include open (blackhead) and closed (whitehead) comedones. Inflammatory acne lesions include papules, pustules, nodules and cysts; their characteristics are further listed in *Table 11*.

Table 11. Characteristics of acne lesions.

Acne lesion	Description	References
Comedone	Clogged hair follicle, can be open or closed	Cunliffe and Gollnick, 2001
Papule	Solid elevation of skin, with no visible fluid, less than either 5 or 10 mm at the widest point	Fitzpatrick *et al.*,2005 James *et al.*, 2006
Pustule	Small elevation of the skin containing cloudy or purulent material	Callen *et al.*, 2000 James *et al.*, 2006
Nodule	Morphologically similar to a papule, greater than either 5 or 10 mm in both width and depth	Callen *et al.*, 2000 James *et al.*, 2006
Cyst	Epithelial-lined cavity containing liquid, semi-solid, or solid material	Fitzpatrick *et al.*,2005

Both non-inflammatory and inflammatory acne lesions originate from the microcomedone. The microcomedone is the initial acne lesion caused by the hyperproliferation of the keratinocytes in the pilosebaceous unit (see *Figure 17* for structure of pilosebaceous unit). Keratinocytes form the lining of normal sebaceous follicle and the outmost surface of skin (Gollnick, 1991). Acne lesions are mainly present on the face, neck, chest, or back where the pilosebaceous units are most dense (Brown and Shalita, 1998).

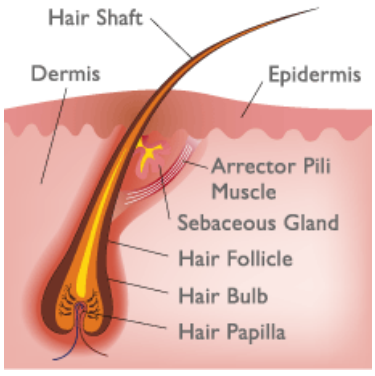


Figure 17. The pilosebaceous unit consists of the hair follicle, hair shaft and sebaceous gland. Source: Singh *et al.*, 2000.

Acne is broadly classified into mild, moderate and severe. Patients with mild acne only have less than 20 comedones and a few papules/pustules. Patients with moderate acne have 10-40 comedones and an equal amount of papules/pustules. Patients with severe acne have more than 40 comedones and

papules/pustules; they also suffer from the presence of nodules and cysts. Moderate and severe acne can cause scars (Andreas, 2010).

3.3.2 Quality of life patients

The psychological aspect of acne should not be neglected (Koo, 1995). Patients often suffer from emotional impairment due to the disfigurement caused by acne regardless of the severity, although there is no significant difference in personal character between affected and healthy individuals (Kenyon, 1966; Picardi *et al.*, 2000). Just as well, scarring can have a profound psychosocial influence on the patient's life (Andreas, 2010). Maisonneuve *et al*. (1987) interviewed 4,597 acne patients and they found that nearly 70% of them reported psychosocial rejection. This is not only a perspective the patients have. It is reported that 18-30 year old acne patients are more frequently unemployed than individuals with healthy skin (Cunliffe, 1986). Mallon *et al*. (1999) interviewed 111 acne patients on their quality of life with a questionnaire. They reported the same level of social, psychological and emotional problems as patients with chronic disabling asthma, epilepsy, diabetes, back pain or arthritis. This is remarkable because acne patients do not think they are ill. However, their emotional and social problems level with that of patient with serious illnesses. Affected individuals have less depression as compared with depressive disorder patients (Niemeier et al., 1998), but they are as depressed as hospitalized psoriasis patients (Gupta and Gupta, 1998). Gupta and Gupta (1998) studied the psychiatric comorbidities of acne patients in 2001 and suggested that it is necessary to assess them for suicide risk.

3.3.3 Causes

The factors that trigger acne are: follicular hyperkeratinisation, *Propionibacterium acnes,* increased seborrhoea and inflammation.

Follicular hyperkeratinization

Keratinocytes are normally shed as single cells and removed with the sebum (Cunliffe and Gollnick, 2001; Gollnick, 1991). Hyperproliferation and reduced shedding of keratinocytes can block the sebum flow and lead to the comedone formation (Cunliffe *et al*., 2000; Gollnick *et al*., 2003). Several factors may contribute to comedone formation, including local androgens, retinoids, local cytokines, changes in sebaceous lipids composition and comedone cycling (Dessinioti, 2010). Comedones undergo cycling comparable to the hair follicle: growth, regression, resting, shedding and growth again (Stenn and Paus, 2001).

Keratinocytes lining the sebaceous follicle express a higher level of 5α-reductase than epidermal keratinocytes. The enzyme 5α-reductase converts testosterone to the more potent androgen dihydrotestosterone (DHT). High levels of DHT can influence comedone formation (Thiboutot *et al*. 1997; Thiboutot, 2002).

Comedones can resolve spontaneously. Aldana *et al*. (1998) found that proliferation and cell cycle markers are expressed differently in comedones compared to sebaceous follicles. This suggests that comedone cycling has an important role in the development and resolution of comedones.

Propionibacterium acnes

P. acnes is a lipophilic and anaerobic bacteria that belongs to the normal skin flora (Lovejoy, 1911). It only proliferates on sebum-rich and low-oxygen area, in this way, blocked sebaceous follicle with

increased sebum secretion provides a perfect environment for its proliferation (Farrar and Bojar, 2010).

P. acnes contributes to the initiation of inflammation in acne, however its presence is not necessary. Leeming (1988) found this bacteria in 68% of one-day-old acne lesions and in 78% of three-day-old lesions. Application of this bacteria to the unaffected skin of acne patients led to inflammation and pustule formation (Strauss and Kligman, 1960; Fleming, 1909). Now it is known that *P. acnes* produces a number of metabolites that induce the innate immune response by activating the TLR2 receptor on keratinocyte, leading to the release of pro-inflammatory cytokines like IL-8 (Pivarcsi *et al.*, 2003). In addition, it produces various exogenous proteases which can interact with protease-activated receptor-2 (PAR-2) on keratinocytes thereby the secretion of pro-inflammatory cytokines increases and leads to inflammatory acne lesions (Lee *et al.*, 2010). On the other hand, Shaheen and Gonzalez found that 10% of one-day old pustules were sterile, while all the 3-day old pustules were colonized, suggesting that microbe colonization is not required for initiation of inflammation (Shaheen and Gonzalez, 2011). Moreover, Leyden *et al.* (1975) found no correlation between *P. acnes* density and severity of inflammation, and Leeming *et al.* (1988) were not able to correlate *P. acnes* density to the progression of inflammatory lesions.

A role for *P. acnes* is also suggested in comedogenesis. Farrar and Bojar (2010) found that co-culture of human keratinocytes with *P. acnes* increased keratinocyte IL-1 production in sebaceous follicles, thus inducing comedogenesis.

Increased seborrhoea

Increased seborrhoea means excess secretion of sebum. Acne patients have much higher sebum secretion than healthy individuals and its accumulation is a prerequisite for acne development (Nikkari, 1974; Downing *et al*. 1987). In addition, the sebum composition of acne patient is altered: more free fatty acids, less linoleic acid and the presence of lipoperoxides, such as squalene peroxides, are some of the hall markers of acne (Wertz *et al.*, 1985; Downing *et al*., 1986; Ottaviani *et al*., 2006). The changes in sebum lipid composition may initiate a previously undiscovered inflammatory cascade early in the development of acne lesions.

Diet may have an influence on sebaceous lipogenesis. The linoleic acid level is low in comedones, which may be associated with comedogenesis (Zouboulis, 2010). Since our body cannot synthesise linoleic acid, diet is the only source. Downing (1972) and Pochi (1970) observed that low glycaemic load diets and extreme caloric restriction can reduce sebum excretion rate; this effect was reversed when normal diet was resumed.

Lipoperoxides, mainly peroxides of squalene (which is a characteristic component of skin sebum) can alter the proliferation and differentiation of keratinocyte thus causing comedone formation (Ottaviani *et al*., 2006).

Sebum production is hormone dependent (Pochi *et al*., 1965; Pochi *et al*., 1974). Adrenal androgens, such as dehydroepiandrosterone (DHEA), have been shown to be the major factor that determines sebum generation and production during the pre-pubertal period (Lucky, 1988; Stewart *et al*., 1992). In a study by Stewart *et al*. (1992), pre-pubertal girls with acne showed higher levels of DHEA than the control group. DHEA can be converted to the more potent androgens testosterone and dihydrotestosterone (DHT), which interact with the nuclear androgen receptors in the sebaceous

follicle (Hay and Hodgins, 1974; Hay and Hodgins, 1978); however, it is not clear how exactly androgens act on sebocyte lipogenesis (Cunliffe and Shuster, 1969).

Retinoic acids like 13-*cis* retinoic acid, 9-*cis* retinoic acid and all *trans*-retinoic acids are able couple to their receptors expressed in human sebocytes. This inhibits the sebocyte differentiation and lipid synthesis and shows an anti-proliferative effect (Zouboulis, 1991; Tsukada *et al*., 2000).

Inflammation

The inflammatory stage is given a lot of attention as the lesions (papule and pustule) may be painful and lead to scarring (Cunliffe, 1998). Recent studies have shown that early inflammatory response is present at the very beginning of the acne lesion development, thus, efforts have been made to reclassify acne as chronic inflammatory disease (Tanghetti, 2013). Norris and Cunliffe (1988) examined early acne lesions and found that lymphoid perivascular infiltration was one of the earliest histological changes occurring its formation. In a study by Jeremy *et al*. (2003) the unaffected skin of acne patients was compared with the skin of healthy individuals. It was found that the unaffected follicle of acne patient is surrounded by large numbers of CD4+ cells and microphages, whereas neutrophiles had totally disappeared, suggesting an initiation of an antigen-specific immune response.

Pro-inflammatory cytokines promote comedogenesis

Pro-inflammatory cytokines promote comedogenesis by inducing the remodelation of pilosebaceous units (Guy *et al*., 1996a; Guy *et al*., 1996b). Interleukin 1 (IL-1) is a pro-inflammatory cytokine that plays a central role in regulating the immune response: skin (hair folicle, sebaceous gland and keratinocyte) can produce IL-1 and serves as a pool for releasing it when needed (Anttila, *et al*., 1992; Boehm *et al*., 1995; Ingham *et al*., 1998), therfore induces inflammation. The expression and secretion of IL-1 significantly increases during the early acne lesion development (Ingham, 1992). Guy *et al*. (1996a) cultured pilosebaceous units with IL-1, which resulted in hyperkeratinization of the follicle. This is very similar to what is observed in the comedogenesis and it can be counter-acted by IL-1 receptor blocker. In another study, Guy e*t al*. (1996b) found that applying epidermal growth factor beta (EGFβ) to cultured pilosebaceous units disrupted the organization of keratinocytes in the infundibulum causing a rupture that it similar to that seen in more severe acne. This suggests EGF may play a role in the process.

IL-8 significantly induced in inflammatory lesion

Trivedi *et al*. (2006) compared the gene expression profile of inflammatory lesions to that of uninvolved skin from the same patients and healthy individuals. They found 211 upregulated genes, most of them involved in matrix remodelling and inflammation. Among the upregulated genes, IL-8 gene expression was increased 52-fold. IL-8 plays an important role in promoting innate immune response in the inflammation site, namely pilosebaceous units (Trivedi *et al*., 2006). One possible cause of the high IL-8 expression may be *P. acnes*, which activates TLR2 and TLR4 (Nagy *et al*., 2006).

Studies have recognized other inflammatory factors, i.e. pro-inflammatory peptidases, and neuropeptides, as well as the emerging role of neuropeptides and sebaceous lipids in inducing inflammation. These are further explained below.

Proinflammatory peptidases in the sebaceous gland apparatus

Pro-inflammatory peptidases may contribute to inflammation in acne. These are ubiquitously expressed enzymes. Beyond their proteolytic function, they also affect many essential biological processes like growth, differentiation, apoptosis, adhesion, motility, and cell to cell interaction. Dipeptidyl peptidase IV (DP-IV) and amino-peptidase N (APN) influence growth, cytokine production and T-cell function; they are both expressed in sebocytes and keratinocytes and are upregulated in hyperproliferative skin disorders (Thielitz *et al*., 2006).

Thielitz *et al.* (2006) investigated the expression and functional relevance of DP-IV and APN in the peripheral blood mononuclear cells (PBMCs) and keratinocytes which show an altered phenotype in early acne lesions. Their results demonstrated that the inhibition of DP-IV and APN can suppress proliferation, enhance terminal differentiation of sebocytes, upregulate the expression of IL-1 receptor antagonist in sebocytes and keratinocytes. Furthermore, the inhibitor turned the *P. acnes*-stimulated T-cells to an anti-inflammatory phenotype by enhancing the expression of the immunosuppressive cytokine TGF-B1 *ex vivo.*

Neuropeptides

Neurogenic inflammation is caused by the local release of inflammatory mediators, such as neuropeptides, from the peripheral terminal of sensory neurons (Geppetti, 2008). Changes in the expression of neuropeptides have been identified during the early stages of acne. Corticotropin-releasing hormone (CRH) is involved in stress response and is also an inflammation mediator. It enhances IL-6 and IL-8 production in cultured sebocytes (Ganceviciene *et al*., 2009a) and its expression is increased in the sebocytes of acne-involved skin (Toyoda, 2002a; Ganceviciene *et al*., 2009b). Immunoreactive nerve fibers produce SP. SP expression is upregulated in the sebaceous glands of acne patient. This is rarely seen in the healthy skin (Toyoda, 2002b). SP is thought to be involved in local neurogenic inflammation. It may play a central role in the pathogenesis of stress-related acne (Toyoda, 2002b; Lee *et al*., 2008).

Sebaceous gland lipids can have pro-inflammatory effects

Acne patients produce more sebum than individuals with healthy skin. Recent studies indicated the tight association of increased sebum secretion and the induction of inflammation, which may be responsible for the initiation of the acne lesion (Zouboulis *et al*., 2005). Besides the increased sebum production, the altered sebum composition may also lead to pro-inflammatory cytokine production in the sebocytes and keratinocytes (Kurokawa *et al*., 2009). The decreased level of linoleic acid in acne patient may lead to increased permeability of comedonal wall (the wall of comedonal hair follicle) to pro-inflammatory substances (Letawe *et al*., 1998; Picardo *et al*., 2009).

Lipid peroxides may be responsible for inflammatory changes in comedones

Acne patients are thought to be under increased oxidative stress, both at the cutaneous level and systemically, compared to healthy individuals (Bowe and Logan, 2010). They also have significantly higher peroxidation levels of sebum, which is able to induce inflammation in acne (Ottaviani, 2006). Squalene is a characteristic human sebaceous lipid that can be easily oxidated. The mono-peroxidation product is primary squalene peroxide on human skin (Bowe and Logan, 2010; Ottaviani *et al*., 2010). An *in vitro* experiment demonstrated that sqaulene peroxide, beyond its role in keratinocyte proliferation, may present pro-inflammatory activity by inducing the expression and secretion of cytokine IL-6 from keratinocytes (Ottaviani *et al*., 2010).

3.3.4 Treatments

Only about 17 to 22% of acne patients used medical therapy (Korczak, 1989; Poli *et al*., 2001). Recently, Nijsten *et al*. (2007) examined 594 adolescents for acne in Netherlands: 13% used topical treatment and 5% used systemic drugs; mainly women and patients with more lesions tend to use acne therapy (Nijsten *et al*., 2007). An earlier study found that 86% of the patients think that face washing with soap is enough to cure acne. Smithard *et al*. (2001) interviewed 317 pupils in UK and reported that the most popular way of dealing with skin problem is increased cleansing (82%) and increased water intake (50%); only 9% had used over-the-counter skin care products and only 3% had used prescribed drugs.

Topical

Several topical acne treatment are available. Topical treatment of acne using retinoids has become more and more popular, as retinoids can suppress comedone formation and lead to comedolysis (dissolution of pore impaction). It also regulates the immune response (Bikowski, 2004; Ramanathan and Hebert, 2011). Retinoids are used as a basic treatment for all types of acne: mild, moderate and severe (Gollnick *et al*., 2003; Strauss *et al*., 2007). Several types of topical retinoids are used including tretinoin (all *trans*-retinoid acids), isotretinoin (13-*cis* retinoid acid, not allowed the United States), adapalene, and tazarotene (not available in the United Kingdom) (Thielitz and Gollnick, 2008). Local side effects of these compounds are skin irritation including erythema, scaling, burning, and dryness (Chivot, 2005; Thielitz, 2008; Thielitz and Gollnick, 2008; Ramanathan and Hebert, 2011). Furthermore, retinoic acid play an important role in embryonic development, thus, administration of retinoid at a high dose is teratogenic. For this reazon the use of retinoids is contraindicated during pregnancy and breastfeeding (Thielitz, 2008). Women of child bearing age using topical retinoids should be aware of the need for contraception (Williams *et al*., 2012).

Three main types of antibiotics are used to treat acne topically: clindamycin, erythromycin and cyclines. among these, clindamycin and erythromycin are the most popular (Tan, 2004). Antibiotics are not very suitable for resolving the comedone. The main target of topical antibiotics are inflammatory lesions and they are used for mild to moderate acne. (Abad-Casintahan, 2011). Bacterial resistance due to antibiotic use has become a growing concern (Thiboutot, 2009). Several studies found that the increasing antibiotic resistance of *P. acnes* correlates with the increasing use of antibiotics (Purdy, 2006; Leyden *et al.,* 2009; Thiboutot, 2009). Erythromycin is the most effective topical antibiotic on inflammatory lesion (Dreno, 2010). The efficacy of this antibiotic has significantly dropped over years from 1980 to 2000 (Simonart and Dramaix, 2005). This means that it is not recommended to use as monotherapy. Topical antibiotics should be used in combination with retinoic acid or benzoyl peroxide to shorten the duration of treatment and avoid bacterial resistance (Dawson and Dellavalle, 2013).

Benzoyl peroxide is used as a treatment for mild to moderate inflammatory acne. It is a non-antibiotic bactericide that kills bacteria by producing free radicals in the hair follicles (Gamble *et al*., 2012). It can be used in combination with antibiotics as combined treatment to minimize bacterial resistance (Elston, 2009). Benzoyl peroxide also has anti-inflammatory properties: it inhibits the release of ROS from neutrophils, thus inhibiting the inflammatory response (Hegemann, 1994). Dose-dependent irritating effects on benzoyl peroxide treated skin include redness, desquamation and burning sensations (Mills, 1986). Benzoyl peroxide can cause allergic contact dermatitis in rare cases (about 1:500). Furthermore, it is highly oxidative so patients using it should be counselled about its

abilities of bleaching brilliant or dark coloured clothing, bedding and even hair colour (Gamble *et al*., 2012).

Oral

Systemic agents are used for moderate to severe acne that does not respond adequately to topical treatment. Hormonal therapy is recommended only for (non-pregnant) female patients (it cannot be applied to men, since it can cause feminization) with moderate to severe acne and hyperandrogenism (Katsambas, 2010). The hormonal therapy can be very effective regardless of the serum androgen level (George, 2008). High levels of androgens can increase the secretion of sebum, which cause comedogenesis (Zouboulis, 2004). Hormonal agents work as androgen receptor blocking or suppressing the androgen production by the ovary or adrenal gland (anti-androgen) thereby opposing the effect of androgens on hair follicle (Dessinioti and Zouboulis, 2010).

Systemic antibiotics can be used to treat moderate to severe acne (Gollnick, 2003). Only antibiotics that can reach the follicular duct of the sebaceous follicles colonized by *P. acnes* can be used in oral antibiotic treatment. Besides the antibiotic effects, their anti-inflammatory effects also play an important role in the acne treatment (Skidmore, 2003). One of the major drawbacks of oral antibiotic is bacterial resistance. Antibiotic treatment failure is strongly associated with the presence of *P. acnes* resistance. Combined therapy (e.g. with topical retinoids) should be used to minimize the resistance and improve efficacy (Eady, 2003). Several adverse effects of antibiotics are found. Gastrointestinal disturbances and vaginal candidiasis are the two most common side effects shared by all oral antibiotics against acne (Ochsendorf, 2010).

The retinoid isotretinoin is notably efficient for severe acne; it is thought to target all four underlying factors of acne by exerting anti-inflammatory effects, decreasing sebum production, normalizing follicular desquamation, and suppressing the hyper colonization of *P. acnes* (Dawson, 2013). Pregnant women should not use isotretinoin because of its teratogenic effects. Careful consideration should be given to teenagers from 12-17 on the use of isotretinoin, especially for those have metabolic diseases or bone diseases (Jonathan Wilkin, 2010). Other adverse effects of isotretinoin include dry eyes, chapped skin, myalgias, epistaxis, and changes in transaminase concentrations and serum lipid, most of which disappear after stop using isotretinoin (Dawson, 2013).

3.3.5 Summary acne

- Acne is a common skin condition that mainly affects teenagers
- Non-inflammatory acne lesions include open and closed comedones; Inflammatory acne lesions include papules, pustules, nodules and cysts
- Factors that trigger acne are follicular hyperkeratinisation, *Propionibacterium acnes,* increased seborrhoea and inflammation
- *P. acnes* only proliferates on sebum-rich and low-oxygen areas; blocked sebaceous follicles with increased sebum secretion provide a perfect environment for its proliferation
- *P. acnes* produces a number of metabolites that induce innate immune response
- Changes in sebum lipid composition may initiate inflammatory cascade in the early development of acne lesions
- Topical treatments include retinoids, antibiotics and benzoyl peroxide. Oral treatments include hormonal therapies, oral antibiotics and isotretinoin

3.4 Atopic dermatitis

3.4.1 Symptoms

Atopic dermatitis (AD), also called eczema, is a common skin disease. It affects roughly 6% of the population in the United States (Hanifin and Reed, 2007) and is on the rise in developing countries (Williams *et al.*, 2008). It is often associated with other atopic disorders such as asthma and allergic rhinitis (hay fever). Children with AD showed a 25.1% prevalence to develop asthma compared to 12.3% in children without AD in the United States (Hanifin and Reed, 2007). AD is caused by a dysfunctional skin barrier function and an imbalanced immune system (Bieber, 2008). These factors are influenced by genetics (Morar *et al.,*2006; Palmer *et al.,* 2006), the environment (Silverberg *et al.,* 2013) and the mental health of the patients (Schmid-Ott *et al.*, 2001a; Schmid-Ott *et al.,* 2001b). The disease changes with age. The general symptoms per age group are as follows:

- Infants (0-2 years): Red papules and vesicles on the cheeks, scalp or forehead. These bumps are extremely pruritic, which means they are very itchy.
- Children (2 years to puberty): Papules and plaques that are lichenified, which means the epidermis is significantly thickened and has highlighted skin lesions. These papules and plaques are also highly pruritic and can be found on the wrists, ankles, feet, hands, frontal side of the elbow and the back of the knees.
- Adults (puberty and up): Bigger lichenified plaques and papules that are red due to increased blood flow and are highly pruritic. The lichenified plaques grew by the chronic damage to the skin. The affected zone grows what is seen in the children stage to include the face, neck, upper arms, back, fingers and toes (Akdis *et al.,* 2006).

The pruritic properties of the affected skin continues through the day and increases at night, which can cause loss of sleep. Combined with the visibility of the disease, this can dramatically impact the quality of life of the patient (Bieber*,* 2008).

Atopic dermatitis has three stages (see *Figure 18*): **non-atopic dermatitis**, **"True" atopic dermatitis** and **autoallergic atopic dermatitis** (Bieber, 2008)**.** Non-atopic dermatitis is a non-IgE associated form of AD. However, it can transform into "true" atopic dermatitis due to the development of sensitization of the skin to IgE. "True" atopic dermatitis can develop into autoallergic atopic dermatitis by scratching. Scratching damages the skin cells, which releases auto antigens. These autoantigens induce IgE autoantibodies which make the inflammatory response worse (Altrichter *et al.*, 2008).

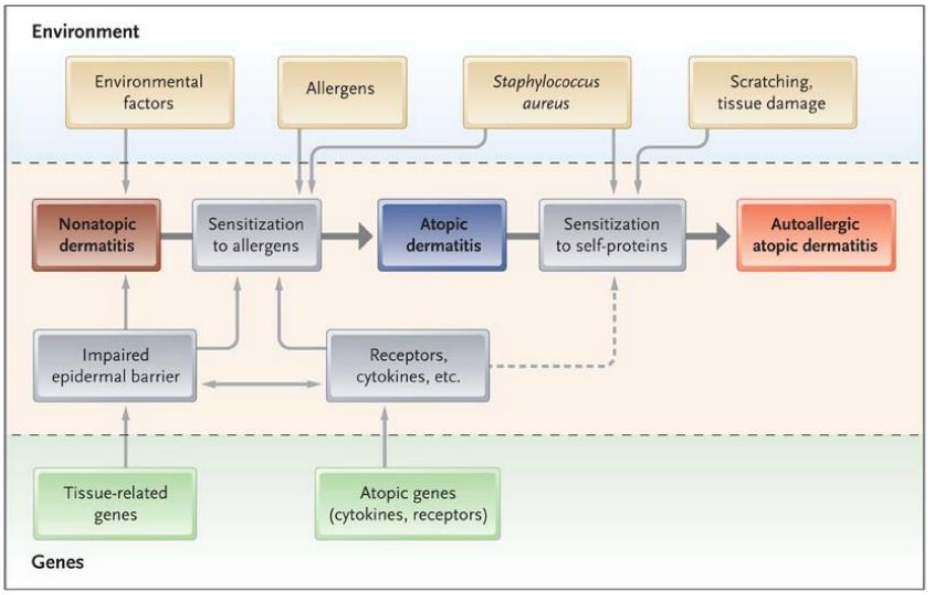


Figure 18. Schematic overview of the development of non-atopic dermatitis into "true" atopic dermatitis and finally autoallergic dermatitis, indicating several environmental and genetic factors contributing to these processes. Source: Bieber, 2008.

3.4.2 Treatments

AD is a multifactorial disease which cannot be treated with only one treatment; multiple treatments are needed to relieve the symptoms. The most important feature of AD is the dry skin, which results from increased water loss from the skin. This means an important part of treating AD is applying a fatty cream to the skin and avoiding degreasing soaps. Irritants like alcohol, chemicals, smoke and dust mite should also be avoided when possible (Leung and Bieber, 2003). Besides protecting the skin and avoiding irritants, AD can also be treated by influencing the immune system.

Treatments with corticosteroids have proven to be effective, but when used in the long term patients can suffer from side effects like skin atrophy (Van der Meer *et al.,* 1999). The corticosteroids suppress the inflammatory reaction, e.g. reducing eosinophils and their products (Beltrani, 1999). Another treatment that affects the immune system is the use of calcineurin inhibitors. These work as immunosuppressors and decrease inflammation. They also decrease the antigen presentation of Langerhans cells and inflammatory dendritic epidermal cells (Wollenberg *et al.,* 2001). However, because the skin is damaged at AD plaques, the side effect is that the inhibitors can enter the blood stream and cause a systemic effect. Long term use of these inhibitors increases the chance of a viral skin infection or skin cancer (Wollenberg *et al.*, 2001).

Emotional stress has a different effect in the skin of AD patients compared to healthy controls. Upon emotional stress, the immune response in AD patients is significantly higher (Schmid-Ott *et al.,* 2001a, Schmid-Ott *et al.,* 2001b).

3.4.3 Genetics and environment

Genetic

Genetic factors are very important in developing atopic dermatitis. In a study of Danish children, monozygotic twins showed a 77% concordance rate, while among dizygotic twins this was only 15%

(Schultz and Holm, 1986 as cited in Strachan *et al.,* 2001). A study in England showed a concordance rate of 49% in monozygotic twins against 24% in dizygotic twins (Strachan *et al.,* 2001).

Impairment of the skin can have a significant effect on the development of AD, and genes are an important factor in developing this impaired skin. (Irvine and McLean, 2006; Palmer *et al.,* 2006).

Certain genes have been found to be linked to the allergy aspect of AD. Young *et al.* (2000) reported there is significant evidence to link a locus, which is on chromosome 3q21, to allergic sensitization. Linkage was also found with chromosomes 1q21, 17q25 and 20p. These loci are associated with dermal inflammation and immunity (Cookson *et al.,* 2001). These studies indicate that a predisposition towards allergic sensitization is a genetic factor that can influence the development of AD.

Environment

The impact of the environment was shown in a study by Matsuda *et al.* (1997). NC/Nga mice, which histologically and clinically have very similar skin lesions compared to humans, showed AD like lesions after 8 weeks in a normal non-sterile environment. NC/Nga mice that were placed in a specific pathogen-free environment did not develop these lesions.

Population based studies show that children in smaller families are more prone to develop AD. AD is mostly prevalent in the Western developed countries, especially in urban areas (Taylor *et al.,* 1984; Leung and Bieber, 2003). However, the occurrence of AD is rising in developing countries, in particular in young children (Williams *et al.,* 2008). This phenomenon was explained with the hygiene hypothesis. The hygiene hypothesis states that if there is not enough exposure to pathogens, the balance between Th1 and Th2 would be more Th2 favoured. This imbalance would predispose children to a Th2 allergic response (Leung and Bieber, 2003). However this hypothesis oversimplifies the complex origin of the imbalance. New studies show that it is more probable that non-pathogenic microbial stimuli of a recurrent nature are the cause of the imbalance. In other words: the microbiome that the child comes into contact with does not have to be pathogenic, but does have to occur frequently. This would explain the higher risk of AD in children that were given antibiotics. These children had their microbiome disrupted and decreased the exposure to non-pathogenic bacteria. A few smaller studies have show the potential of probiotics in preventing AD. This also fits with the newly stated hypothesis (Flohr *et al.,* 2005).

3.4.4 Important factors in atopic dermatitis

Skin barrier

A major cause of AD is a dysfunctional skin barrier (Bieber, 2008). As described in *3.1.1 The skin*, the outer layer of the skin is the cornified layer. The differentiation of keratinocytes into corneocytes is closely regulated to meet the shedding of the corneocytes to keep homeostasis (Candi *et al.,* 2005). Because this complex process requires the expression of certain genes at certain moments and is so tightly regulated, it can be easily disrupted by gene mutations. A vital protein in the formation of the cornified envelope is filaggrin (see also *Figure 12*). Two independent loss-of-function genetic mutations in the filaggrin (FLG) gene have been found to be strong predisposing factors for developing AD (Palmer *et al.,* 2006). In a study with Irish childhood eczema at least 47% of the individuals carried one or more null FLG mutations (Sandilands *et al.,* 2009). Profilaggrin is cleaved into filaggrin proteins which is critically for arranging the CE structure. The malfunctioning of filaggrin

is detrimental for the water holding capacity of the skin (Candi *et al.,* 2005). These findings indicate that a dysfunctional filaggrin gene can have severe consequences for the skin barrier.

A major cause of AD is a dysfunctional skin barrier (Bieber, 2008). As described in *3.1.1 The skin*, the outer layer of the skin is the cornified layer. The differentiation of keratinocytes into corneocytes is closely regulated to meet the shedding of the corneocytes to keep homeostasis (Candi *et al.,* 2005). Because this complex process requires the expression of certain genes at certain moments and is so tightly regulated, it can be easily disrupted by gene mutations.

A vital protein in the formation of the cornified envelope is filaggrin (see also *Figure 11*). Two independent loss-of-function genetic mutations in the filaggrin (FLG) gene have been found to be strong predisposing factors for developing AD (Palmer *et al.,* 2006). In a study with Irish childhood eczema at least 47% of the individuals carried one or more null FLG mutations (Sandilands *et al.,* 2009). Profilaggrin is cleaved into filaggrin proteins which is critically for arranging the CE structure. The malfunctioning of filaggrin is detrimental for the water holding capacity of the skin (Candi *et al.,* 2005). These findings indicate that a dysfunctional filaggrin gene can have severe consequences for the skin barrier. Another important part of the skin barrier is the lipid envelope. The lipid envelope is important for reducing water loss by amongst others retaining NMF in the corneocytes. Decreased levels of NMF are a feature of moderate to severe AD. Filaggrin is the most important modifier of NMF but severe AD can also affect NMF (Kezic *et al.,* 2011).

Ceramides are important structural components of the lipid envelope. Ceramide metabolism is altered in AD patients (Meckfessel and Brandt., 2014). For instance, in AD patients there is a significant reduced amount of ceramide 1 (Yamamoto *et al.*, 1991). Basic ceramides are produced by conjugating a sphingoid base and a fatty acid via an amide bond. One such sphingoid base is sphingosine (Meckfessel and Brandt, 2014). Sphingosine has a strong antimicrobial effect on *S. aureus. S. aureus* also plays an important role in worsening symptoms of AD, as is explained later. In AD patients, sphingosine levels were significantly downregulated in the upper *stratum corneum*, this is not only the case in involved skin (AD lesions) but also in uninvolved skin (Arikawa *et al.*, 2002). A decreased level of sphingosine may therefore be important in colonization resistance against *S. aureus*. A possible underlying mechanism for the decrease in sphingosine levels may be reduced activity of acid ceramidase. Ceramidases break down ceramides, thereby generating sphingosine (Arikawa *et al.*, 2002).

Another indication that the cornified layer is different in AD patients was shown by Tabata *et al*. (1998). They showed that an anionic soap had an disruptive effect on the cornified layer. The soap caused increased amounts of transepidermal water loss in AD patients compared to healthy patients. There was also an increase in the reaction of the immune system, eosinophils were found in the perivascular liquid of AD patients but not in the healthy patients. This shows that the skin of people with atopic dermatitis is more vulnerable to the use of soap.

TRPV1

The TRPV1 (transient receptor potential vanilloid subfamily member 1) receptor is highly expressed in epidermal keratinocytes and nerve fibres in the skin (Inoue *et al.,* 2002). The receptor is activated by acid heat and capsaicin, an ingredient found in hot peppers (Suh and Oh, 2005). A study by Denda *et al.,* (2006) showed that TRPV1 activation delayed skin barrier recovery. Inhibition of TRPV1 resulted in a quicker recovery of the skin in hairless mice and humans compared to skin which was

not treated with a TRPV1 inhibitor. TRPV1 is upregulated in the lesions of AD patients. (Yun *et al.,* 2011) A potent TRPV1 antagonist is PAC-14028. In a study by Yun *et al*. (2011) with AD mouse models, PAC-14028 significantly recovered the loss of intercellular lipids in the lipid envelope, which in turn reduces transepidermal water loss. PAC-14028 also restored the balance in the position where loricrin and filaggrin are expressed in the epidermis. These changes repaired the skin and therefore impaired the potential of allergens to penetrate the skin to feed the inflammation. This resulted in a decrease in IgE expression and degranulation of the mast cells compared to AD mouse models which did not receive the TRPV1 inhibitor. Besides its skin restoring effect PAC-14028 reduced scratching behaviour in the AD mouse model, which could be linked to the role TRPV1 plays in the itch signal (Yun *et al*., 2011).

Immune system

The exact onset of AD is unknown, because it can have multiple causes. The initial inflammation can be caused by scratching, which releases pro-inflammatory cytokines, by an IgE mediated allergic reaction to food or aeroallergens or by a non-IgE allergic reaction (Bieber, 2008). The IgE mediated allergic reaction becomes more stronger every time it is induced (Spergel *et al.,* 1998). This means that the patients become sensitized to an allergic reaction (see *Figure 19*).

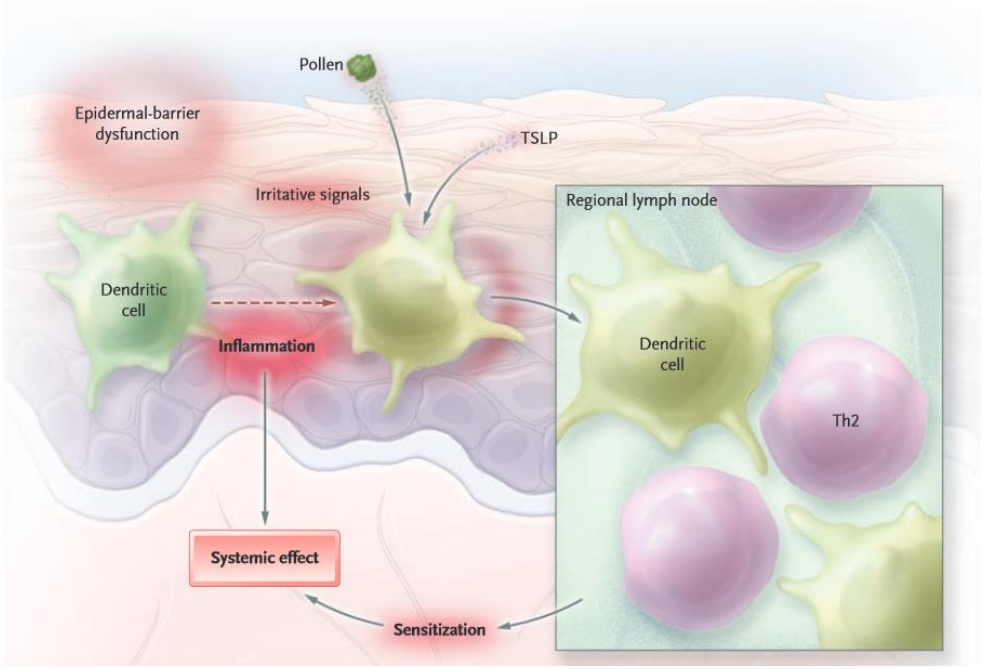


Figure 19. The figure shows the beginning of an allergic reaction to allergens causing sensitizing of the immune system to the same allergen. The allergen is able to pass through the dysfunctional skin barrier and activates the dendritic cells. The dendritic cells in turn activate the Th2 cells. This causes an allergic reaction. If the same allergen reaction is induced multiple times, sensitization occurs and the allergic reaction will become stronger. Source: Bieber, 2008.

There are two populations of epidermal dendritic cells: Langerhans cells and inflammatory dendritic epidermal cells (IDECs). These cells both express the IgE high affinity receptor FcERI (Bieber *et al.,* 1992). IgE binds to these receptors and causes the dendritic cells to take up and present allergens to the Th cells. The Langerhans cells activate Th2 cells, whereas the inflammatory dendritic epidermal cells are recruited in reaction to the inflammation caused by the allergens and activate the Th1 cells.

The inflammatory dendritic epidermal cells migrate to the inflammation site within 72 hours (Kerschenlohr *et al.,* 2003).

The inflammation in AD takes place in two phases (see *Figure 20*). It starts with the binding of IgE with allergens to the Langerhans cell. This cell activates Th2 cells and produce IL-16 and MCP-1 (monocyte chemotactic protein 1). The Th2 cells begin to produce IL-4, IL-5, IL-13 and IL-31. These interleukins initiate the allergic reaction. After this initial stage the MCP-1 and IL-16 attract monocytes to migrate into the skin tissue. The IDECs secrete IL-12 and IL-18 which changes the type of inflammation from a Th2 dominant inflammation to a Th1 dominant inflammation (Bieber, 2008). The IFNy produced by Th1 cells in the second phase of inflammation has been suggested to cause apoptosis in keratinocytes, further disturbing the skin barrier (Trautmann *et al.*, 2000). Tregs which normally inhibit the immune response are absent from AD lesions (Verhagen *et al.*, 2006). Eosinophilia is seen as a hallmark of atopic diseases (Beltrani, 1999). In AD there is an increased amount of eosinophils in AD lesions (Leung, 2000). These eosinophils produce ROS intermediates and release toxic granule proteins (Gleich, 2000), thereby further compromising the skin. Eosinophils that are activated by IL-4 and IL-5 produce IL-12, which promotes the switch from the Th2 dominant inflammation to the Th1 dominant inflammation (Grewe *et al.,* 1998).

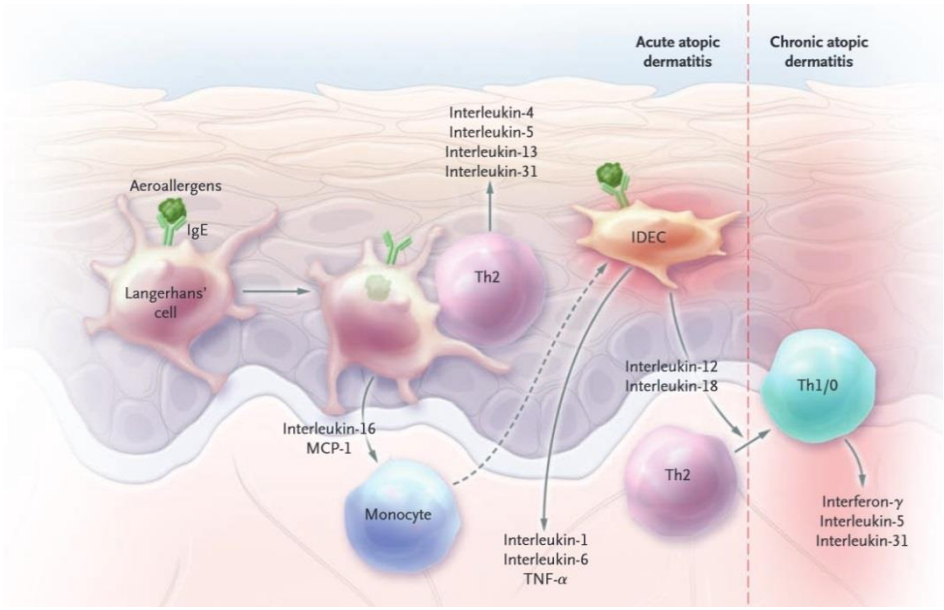


Figure 20. Langerhans cells present the antigens to Th2 cells starting the Acute atopic dermatitis stage. The Th2 cells produce IL-4, IL-5, IL-13 and IL-31. Besides activating the Th2 cells the Langerhans cells also produce IL-16 and MCP-1 which attracts monocytes to enter the inflammatory reaction. The IDECs secrete IL-12 and IL-18 which turns the Th2 dominant inflammation into an Th1 dominant inflammation. This Th1 dominated inflammation is the Chronic atopic dermatitis stage. In which Th1 cells produce IFNy, IL-5 and IL-31. Source: Bieber, 2008.

The innate immune system of AD patients also differs from healthy persons: AD patients have significantly less anti-microbial peptides, which increases the chance of getting a bacterial infection (Ong *et al.,* 2002).

Itching

The high pruritic properties of AD can affect the quality of life of the patients significantly. In a study by Yosipovitch *et al.* (2002) which followed 100 patients with AD, pruritus appeared in 87% of

patients on a daily basis. The most itchy body parts were the back of the knees (83%), the front of the elbows (76%) and the neck (65%). This corresponds with the areas were the symptoms of AD appear in adults. Daily factors that increase the intensity of pruritus were sweat (96%), physical effort (73%), dryness of the skin (71%) and stress (71%). These factors match the results of a study done with psoriasis patients. Daily factors that increased pruritus in psoriasis patients were skin dryness (80%), ambient heat (81%), sweating (65%) and stress (55%) (Yosipovitch *et al.,* 2000). To indicate the intensity of the pruritus they compared it to the moment when a mosquito bite is at its strongest. In the most intense moment of pruritus in AD the intensity was almost twice as high as the mosquito bite (Yosipovitch *et al.,* 2002).

The itchiness increases at night and can cause sleep disturbances. In a study by Bender *et al.* (2003) measured with actigraphs, the time in bed between patients and control was no different. However, AD patients awoke more often and when they woke up it took them longer to fall back asleep. This results in an lower sleep efficiency than healthy people. This matches the sleep diaries the groups had to make, the AD patients reported lower sleep quality and more awakening.

Although the exact mechanisms by which the intensity of pruritus increases during the night are unknown, certain factors that play a role in this process have been revealed (Patel *et al.,* 2007). An increase in body temperature may be a factor, because increased ambient temperature is associated with increased pruritus while a cold ambient temperature is found to reduce the intensity of pruritus (Yosipovitch *et al.,* 2002). The skin is more permeable in the evening and night than in the morning. This resulting increase in transepidermal water loss causes the skin to be drier, which increases pruritus (Yosipovitch *et al.,* 1998).

Although itching is a symptom of AD and not its cause, effective prevention of scratching can greatly reduce the symptoms of AD and help in controlling the lesions. Scratching disturbs the skin, *S. aureus* can better adhere to scratched skin, scratching causes water loss which makes the skin dry, makes it possible to go from atopic dermatitis to autoallergic atopic dermatitis and can greatly influence the quality of life of the patient. Thus, preventing itching can be great for improving AD.

Reducing itching

As mentioned previously, TRPV1 is involved in the itch response (see *Figure 21*). The itch response induced by histamine was reduced by knocking out the TRPV1 gene. The itching effect of two other important pruritogens, namely serotonin and ET-1, was absent when the $TRPV1^{+}$ neurons were knocked out (Imamachi *et al.,* 2009). This indicates that TRPV1 is directly involved in the pathway leading in scratching behaviour when triggered by histamine and is present in the neurons that relay the itching signal caused by serotonin and ET-1. Currently PAC-14028 is undergoing clinical trials, because of the accelerated skin repair and anti-pruritic capacities of the TRPV1 inhibitor (Lim and Park, 2011).

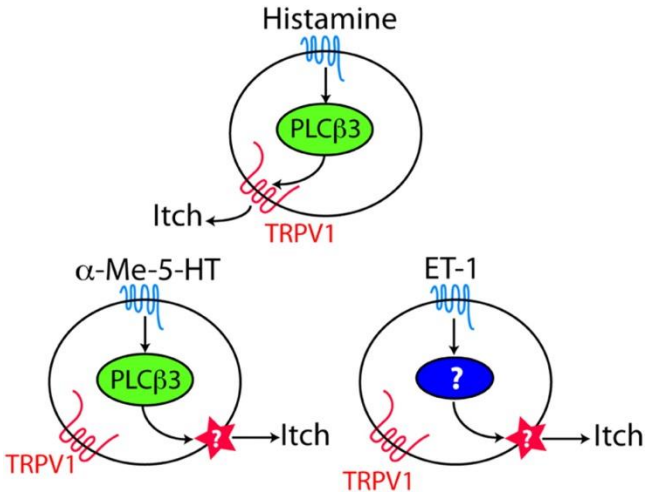


Figure 21. TRPV1 is very important for the itch reaction. The pruritogen histamine triggers TRPV1 which results in the itch response. The pruritogens serotonin and ET-1 do not activate the itch response via TRPV1 but through another system. The exact mechanism is unknown. However TRPV1 is located on the membrane of the cells which induce the itch reaction, indicating that the neurons which express TRPV1 are crucial in the itch response. Source: Imamachi *et al.,* 2009.

Stress

The mental state of patients can greatly affect the symptoms of AD. A study by Gil *et al.* (1987) showed a significant correlation between Problem total (Stressful life events and everyday problems) and AD distress. A cause for the increased symptoms is that stress-induced immunomodulation is altered in patients with AD. When patients were subjected to a stress test there was a significant increase in lymphocytes, neutrophils, basophils and monocytes in the blood compared to healthy people (Buske-Kirschbaum *et al.,* 2002). The significant increase in leukocyte numbers can affect the symptoms of AD. Another study found the same result: after a stress test there was an increase in CD8+ cells and eosinophils (Schmid-Ott *et al.,* 2001a). As previously mentioned, eosinophils play an important role in chronic allergic inflammation and increased numbers are often found in AD patients (Leung, 2000). The stress also induced a significant increase in IL-5 producing CD4+ cells (Schmid-Ott *et al.,* 2001b). IgE was also significantly increased after the exposure to the stress test. This antibody is also an important factor in the overreaction of the immune system. Besides these Th2 associated increases, there was an increase in IFNy levels, which activate the Th1 balance (Buske-Kirschbaum *et al.,* 2002; Schmid-Ott *et al.,* 2001b). This indicates that in existing lesions where there is already a Th1 dominance the symptoms could worsen as IFNy has a variety of effects that enhance the inflammatory reaction. The increased levels of eosinophils and IgE could predispose non-lesion spots to a stronger reaction to allergens.

These studies show that the immune system of patients with AD reacts different to stress than that of healthy persons. A group of young children with AD had a relaxing massage for 20 minutes every day for 1 month combined with standard topical care. This group was compared to a group of children which received the same topical care but no massage. There was a significant difference between the groups; the massaged children seemed less anxious and the symptoms of AD reduced more than those of the non-massaged group (Schachner *et al.,* 1998). Although it was a small study and it did not test for long-term effects it suggests that the mental state of the patient affects the severity of the symptoms.

Staphylococcus aureus

Staphylococcus aureus is found in 90% of AD lesions (Leyden *et al.,* 1974). An indication in why *S. aureus* is so frequently found in AD patients comes from a study by Kisich *et al.* (2008). They showed that AD patients have lower levels of human β-defensin-3 (hBD3) compared to healthy individuals. hBD3 is an anti-microbial peptide produced by the keratinocytes which kills *S. aureus* (Kisich *et al.,* 2007). The down-regulation of hBD3 is due to inhibition by IL-4 and IL-13. These are the Th2 cytokines indicating that an allergic reaction to allergens predisposes the skin to invasion by *S. aureus*.

Scratching is an important factor in disrupting the skin barrier and enhancing the binding of *S. aureus* to the skin. *S. aureus* produces enterotoxin A and B or toxin 1 (Leung, 2003). These substances are called superantigens and amongst others induce IgE-mediated basophil histamine release (Leung *et al.,* 1993). The superantigens amplify the allergic inflammatory skin immune response (Bunikowski *et al.,* 1999). The effects of S. aureus could also lead to glucocorticoid insensitivity (Hauk and Leung, 2001 as cited in Novak *et al.,* 2003).

3.4.5 Summary atopic dermatitis

- Atopic dermatitis is a common skin disease, affecting: infants, children and adults
- Three stage of the disease: Non-atopic dermatitis, atopic dermatitis and auto-allergic atopic dermatitis
- Symptoms depend on age, however the affected skin is highly pruritic
- Genetic predisposition for AD can be found in, an altered immune system or an impaired skin barrier
- Environmental factors can influence the chance of developing AD, e.g. exposure to non-pathogenic bacteria and exposure to allergens
- AD patients have an impaired skin barrier, important factor is mutation in expression of filaggrin or ceramides
- Immune system is altered in AD patients, more prone to inflammation
- TRPV1 is important in skin restoration and itching response
- Itching is a daily factor in the lives of AD patients and can worsen the lesions
- AD patients have a different immune response to stress
- *S. aureus* is found in 90% of the AD lesions and worsens the symptoms by secreting superantigens.

3.5 Market study

Since some pre-, pro- and postbiotics-containing skin care-related products exist currently in the market, we searched information about them on the Internet and directly in shops. By both means we were able to find several products containing pre- and postbiotics and only very few or none containing probiotics. Information found on the internet about products containing probiotics is summarized in *Table 12;* here only one product containing prebiotics was included as an example. In the shops we were not able to find data about products containing probiotics. However, some of the skin care products available indicated the presence of prebiotics and/or postbiotics. These products are not further reported or discussed here since they are not our main focus.

Table 12. Existing (non-oral) probiotic products for the international market.

Name	Company	Ingredients	Claimed mechanism	Application	Reference
TS7® (Liquid, Lotion, cream, mask)	Tensall Bio-tech (Taiwan)	Seven probiotic strains	Promoting skin cell and collagen proliferation. Whitening and moisturizing skin	Skin	Retrieved December 1, 2014 from http://www.tensall.com/en/material.php
RAD-III® (Liquid, Lotion, cream, mask)	Tensall Bio-tech (Taiwan)	Three probiotic strains	Moisturizing sensitive skin and relief skin itching. Providing skin natural essence, protection and repair.	Skin (atopic dermatitis)	Retrieved December 1, 2014 from http://www.tensall.com/en/material.php
HOFF© (Liquid, Essence, Lotion, cream)	Tensall Bio-tech (Taiwan)	Two strains of human gastrointestinal probiotics	Helps recovering normal skin flora, moisturizing and softening dry and hardened skin.	Skin	Retrieved December 1, 2014 from http://www.tensall.com/en/material.php
Sesative© (Liquid, Essence, Lotion, Cream, Mask)	Tensall Bio-tech (Taiwan)	Probiotic strains on Sesame seeds.	Utilizes the essence of sesame seeds for a functional healthy drink and skincare applications.	Skin	Retrieved December 1, 2014 from http://www.tensall.com/en/material.php
Clear skin probiotic moisturizer	Eminence (USA)	Cucumber juice, willow bark extract, **yogurt**, tea tree oil, calendula oil, shea butter, Bio-Complex(antioxidants, vitamins, Alpha Lipoic Acid)	Cucumber and tea tree help prevent the appearance of blemishes and reduce the appearance of inflammation while probiotics exfoliate and soothe the complexion.	Skin	Retrieved December 1, 2014 from https://eminenceorganics.com/us/product/clear-skin-probiotic-moisturizer
Unstress line	Christina Cosmetics (USA)	*Lactobacillus*	Competition with pathogenic bacteria and enhance the natural immune system	Skin	Retrieved December 1, 2014 from http://www.christina-cosmeceuticals.com/products/unstress/science-behind/
Magoroku Skin Care Lotion	Dr. Ohhira Probiotics (Japan)	14 Wild Plants, the probiotic *Enterococcus faecalis* TH10, Aloe Vera, Peach Leaf, Loquat Leaf	It replaces the old oxygen for new oxygen to prevent oxidation and damage to the skin. Inhibits growth of pathogens and breaks down fatty acids	Skin	Retrieved December 1, 2014 from http://drohhiraprobiotics.com/dr_ohirra_probiotic_magoroku_skin_lotion.php
NaturXtra Homecare	Chrisal (Belgium)	*Bacillus* species	Competition with pathogenic bacteria due to direct competition and quorum sensing to inhibit the growth of pathogens	Cleaning	Retrieved December 1, 2014 from http://www.chrisal.com/test-reports.html
Sanamedi allergen spray	Sanamedi (The Netherland	Probiotics	Removes the allergens via probiotics	Homeware, e.g. matrasses,	Retrieved December 1, 2014 from http://www.sanamed

	s)			carpet	i.nl/sanamedi-spray/allergeen-spray
Miracle Mask and Purify cleansing wash	Nude (USA & Canada)	Prebiotics (α-Glucan Oligosaccharide)	This prebiotic supports and protects the skin's delicate balance of microflora. It can also help skin against external stressors.	Skin	Retrieved December 1, 2014 from http://www.nudeskincare.com/ingredients/glossary
Advance renewal overnight repair mask	Nude (USA & Canada)	Probiotics	Probiotics help stabilize microflora on the skin and protect skin from environmental stressors, soothes skin.	Skin	Retrieved December 1, 2014 from http://www.nudeskincare.com/ingredients/glossary

3.5.1 Skin treatment

Tensall bio-tech is a company based in Taiwan and released several products for skin care in liquid, lotion, cream and mask forms. The line RAD-III® contains a combination of three probiotics species for preventing atopic dermatitis on various parts of human skin. The symptoms can be remitted through moisturizing the sensitive and itching skin. The bacteria contained in this products are *Lactobacillus paracasei, Lactobacillus casei* and *Lactobacillus rhamnosus* GG. (Retrieved December 1, 2014, from http://www.tensall.com.tw/big5/material.php?as=28).

The line TS7®, which belongs to Tensall bio-tech, comprises products in liquid, lotion, cream, and mask forms. The formulations contain metabolized derivatives from seven strains of probiotics that enhance the skin cells regeneration and proliferation. They also promote collagen production, whitening and the moisturizing processes. The seven species contained in the TS7® products are *Lactobacillus acidophilus, Bifidobacterium bifidum, Bifidobacterium longum, Lactobacillus casei, Lactococcus lactis, Streptococcus thermophilus,* and *Lactobacillus bulgaricus*. (Retrieved December 1, 2014, from http://www.tensall.com.tw/big5/material.php?as=28).

Another line from Tensall bio-tech is Sesative®, which contains metabolized derivatives of several probiotic strains on Sesame seeds (postbiotics). Consumers can consume the essence of the Sesame seeds as a drink or use it for topical application. More information about the mechanism could not be found, but the claims by the company are promising for the postbiotic components found on sesame seeds (Tensall Bio-Tech, n.d.).

Magoroku Skin Care Lotion, which belongs to the company Dr. Ohhira, is a skin care product that combines 14 wild plants, *Enterococcus faecalis* strain TH10, aloe vera, peach leaf and loquat leaf. It is mainly used to treat diaper rash and other conditions that cause mild or severe reddening of infant skin. The product claims to work with new oxygen emanating from the ingredients to replace the old oxygen in the skin pores. This helps preventing further oxidation and damage of skin cells. At the same time, *Enterococcus faecalis* inhibits the growth of harmful bacteria that cause infections, thus preventing inflammation and skin disorders. Fatty acids essential for cell rejuvenation, such as linolenic acids, are broken down into simpler forms for easier absorption by the skin cells, this ultimately restores the healthy skin (Retrieved December 1, 2014, from http://drohhiraprobiotics.com/dr_ohirra_probiotic_magoroku_skin_lotion.php). The company claims that there has been done research on this lotion but no reference to the study can be found on the site.

The line *Unstress* from Christina Company claims to relief stress skin resulted from sun exposure, pollution, hormonal imbalance and other factors by "boosting the body's natural immune system and cell defense mechanisms" (Retrieved December 1, 2014, from http://www.christina-cosmeceuticals.com/products/unstress/) Irritation, itching, inflammation, edema, excess sebum and premature aging are some of the symptoms for which this treatment is indicated. It offers long lasting protection. The key active ingredients in this line are quintescine, venuceane, ceramides, collaxyl, hyaluronic acid, frangipani milk and probiotics; this latter is composed by the genus *Lactobacillus.* According to the website, the mechanism of action of this bacteria is competition as it "crowds out pathogenic organisms" (Retrieved December 1, 2014, from http://www.christina-cosmeceuticals.com/products/unstress/science-behind/). The products that contain probiotics are: Step 3 - Pro-Biotic Peel, Step 9 - Pro-Biotic Moisturizer, Pro-Biotic Day cream SPF 12 and Pro-Biotic Eye & Neck Day cream SPF 12 (Retrieved December 1, 2014, from http://www.christina-cosmeceuticals.com/products/unstress/salon-products/) Christina Company is a US based company that develops skincare products for healthy but also diseased skin. Each line targets both biological and environmental causes and symptoms for a specific skin condition as well as healthy skin. Christina company has a R&D facility where the products are manufactured, tested and clinically proven. Though, no references to scientific articles were found. Currently there are no distributors in The Netherlands, but the products can be found in nearby countries like Belgium and Germany.

3.5.2 Cleaning products

NaturXtra has a line of products in bodycare and homecare containing probiotics. The body care line consists of hand soap, skin and hygiene spray, mouth hygiene toothpaste, feet spray, bath foam, deodorant for men and women, douche gel and hand gel (Retrieved December 1, 2014, from http://www.naturxtra.nl/probiotic-bodycare.html). The homecare line comprises detergent, all purpose cleaner, floor cleaner, textile spray and laundry detergent (Retrieved December 1, 2014, from http://www.naturxtra.nl/probiotic-homecare.html). NaturXtra is a local distributor for Chrisal, the main company located in Lommel, Belgium. The bacteria they use for their homecare product line are from the *bacillus* species (spore forming and gram-positive) (Verstraete *et al.,* 2007). These species are granted the GRAS (generally recognized as safe) label by the Food and Drug Administration indicating they can be used for human purposes without any hazard.

The company claims they have developed a certain technique in which the probiotics produce spores that are only activated when released from the bottle, in this way they able to survive for a couple of years in the product. The patented SPF (Stabilized Probiotic Ferment) logo on the package guarantees that the product contains the same cleaning potential for 2 years minimum. This is accompanied by the 50 Million Probiotics Per mL logo, which ensures the presence of minimum 50 million microorganisms per millilitre in the product that will last for at least two years after its purchase (Retrieved December 1, 2014, from http://bi-safe.be/site/node/15). If this technique is available and actually working, it might be a convenient way to pack and store the probiotics in skin care products.

Chrisal has connections with the University of Gent and Dr Robin Temmerman, formerly part of the university, is currently working in the company as CEO. The company published a study in cooperation with the University of Gent and the Lokeren General Hospital. The study showed that cleaning with their probiotic infused products decreased the amount of harmful hospital bacteria compared to the normal cleaning regime with disinfectants. The products claim to work via the

mechanisms of competitive exclusion and quorum sensing. “The idea behind Competitive Exclusion is that during the cleaning procedure a layer of probiotic bacteria is placed on the treated surface, therefore immediately occupying the ‘field’, the area treated, with beneficial (good) bacteria.” (Verstraete *et al.,* 2007). Quorum sensing is the communication of bacteria via signal molecules related to population density. The probiotics communicate to the pathogens that the population density is high, this causes the harmful bacteria to go into an inactive metabolic state and wait for a better moment to grow. The hospital study showed that the technique of using probiotics instead of disinfectants to combat the hospital pathogenic bacteria works and has a stable result. However, the study was not published in a peer reviewed journal. This could indicate that the study was not completely objective or that the company did not want to reveal the specific bacteria strain they use for their products. This creates doubts about the actual effectiveness of the product and further research to confirm these claims should be performed.

3.5.3 Allergen removing spray

Sanamedi developed a 100% natural allergen removing spray which works via probiotics and is viable for 2-4 years. The website claims that the spray removes allergens with the use of probiotics, however there was no research to support this claim on the website (Retrieved December 1, 2014, from http://www.sanamedi.nl/sanamedi-spray/allergeen-spray). The company was contacted to ask for further explanation of the product but did not gave more information because it would reveal the “tricks of the trade”.

4. DISCUSSION

Based on the information found in the literature about the mechanisms of maintenance of healthy skin and the pathophysiology of rosacea, acne and atopic dermatitis, we selected mechanisms that we believe could potentially be targeted using probiotics. We attempted to focus mainly on bacteria that normally reside on the skin, as these have a higher chance of survival on the skin. We also examined the current state of probiotics on the market, especially in skin care, to evaluate their feasibility in skin care and the best way to implement them.

Probiotics could work by competition of space or nutrition. It could prevent colonization of pathogenic bacteria. Probiotics can be a protective shield against pathogenic bacteria. However, the bacterial strain used has to be capable of surviving on epithelial cells on the skin. The bacterial strains selected for the gut may not survive on the skin because of different environments e.g. pH, aerobic versus anaerobic. Secondly, probiotics also produce some anti-microbial substances. If these do not induce resistant bacteria they are more desirable to use than anti-biotics. Lastly, probiotics or postbiotics can inhibit inflammatory pathways in epithelial cells. Since inflammation is involved in skin conditions, probiotics could reduce the symptoms (Bowe, 2013).

4.1 Targeting healthy skin

Mechanisms that could be targeted to maintain or improve healthy skin are:

- Bacterial production of beneficial compounds
- Improving skin barrier function

4.1.1 Bacterial production of beneficial compounds

Several compounds that are known to be beneficial to the skin and are commonly used in cosmetics are also produced by bacteria, including hyaluronic acid, sphingomyelinase, lipoteichoic acid, peptidoglycan, lactic acid, acetic acid and diacetyl (Lew and Liong, 2013). Due to time constraints, we only looked further into hyaluronic acid for healthy skin. Lactic acid producing *Lactobacillus* is discussed further in *Targeting rosacea*. Sphingomyelinase is discussed in *Targeting atopic dermatitis*.

Hyaluronic acid is widely used in dermatology because it can improve skin hydration and elasticity, improve wound healing, regulate the production of pro-inflammatory cytokines and increase epithelial defence (Lew and Liong, 2013). Bacteria identified so far that are able to produce hyaluronic acid include *Pasteurella multocida* (Rosner *et al.*, 1992), and group A and group C streptococci such as *Streptococcus zooepidemicus* (Armstrong and Johns, 1997). However, these strains are potentially unsafe for application to the skin. Both *P. multocida* and *S. zooepidemicus* are animal-associated pathogens and can occasionally cause infections in humans as well (Wilson and Ho, 2013; Eyre *et al.*, 2010). Other bacterial strains are genetically modified to produce hyaluronic acid, such as *Bacillus subtilis* 168 (Widner *et al.*, 2005) and *Lactococcus lactis* LL-NAB (Chien and Lee, 2007). The use of genetically modified bacteria in skin care may be prohibited or restricted by law. Furthermore, *Lactobacillus rhamnosus* FTDC 8313 and *Lactobacillus gasseri* FTDC 8131 have been shown to produce hyaluronic acid when cultivated in milk (Lew *et al.*, 2012), but it is unknown whether these bacteria normally reside on the skin. They are both commonly used as probiotics for the gut. Therefore, it is not known if these bacteria could survive on the skin and have the right substrates to produce hyaluronic acid, and it is not certain that application of these probiotics will

result in a lasting effect on the skin. Thus, bacteria producing beneficial compounds for the skin could be a promising topical skin treatment. However, more literature research needs to be done to identify bacteria producing these compounds.

4.1.2 Improving skin barrier function

Probiotics could be used to improve skin barrier function. One enzyme that could help improve the skin barrier is sphingomyelinase. This enzyme generates a number of ceramides and phosphorylcholine from glucosylceramide and sphingomyelin precursors. Ceramides are important for the development of extracellular lipid bilayers in the *stratum corneum* (Lew and Liong, 2013). Mammals also produce sphingomyelinase, but in mammalian cells this enzyme is bound to the membrane, whereas bacterial sphingomyelinase is secreted from the cells into the media (Lew and Liong, 2013). Therefore, bacterial sphingomyelinase could increase ceramide production if applied to the skin. One bacterium producing sphingomyelinase that is generally considered safe is the lactic acid bacterium *Streptococcus thermophilus* (Di Marzio *et al.*, 1999). Topical application of a cream containing sonicated (lysated using sound waves) *S. thermophilus* in healthy volunteers increased the amount of ceramides in the *stratum corneum* of the skin (Di Marzio *et al.*, 1999). The researchers indicated that this could be due to the presence of bacterial sphingomyelinase. This bacterium could also be promising in the treatment of AD (see also *4.4.2 Targeting atopic dermatitis - Decreased ceramide levels*). There may be other safe bacteria that produce adequate levels of sphingomyelinase. However, the only one that has been investigated in relation to the skin so far is *S. thermophilus.*

4.1.3 Summary and recommendation healthy skin

In summary, healthy skin might be maintained or improved using probiotics by adding bacteria that can produce beneficial compounds for the skin to the skin microbiome and by improving skin barrier function. A more specific literature search is needed to find out if there are other bacteria producing these kinds of compounds, that are also safe to apply to the skin and able to survive. Keep in mind that a bacterial lysate of such a bacterium may not be any more advantageous to the skin than just applying the beneficial compounds themselves. Therefore we think it is more promising to aim for improvement of the skin barrier by applying for instance the sphingomyelinase-producing *Streptococcus thermophilus* to the skin. It is unknown whether this bacterium is normally resident on the skin, but this strain is generally regarded as safe. *S. thermophilus* was also found during the market study in TS7® produced by Tensall Bio-tech (Taiwan), along with six other probiotic strains. This means that this probiotic is safe for use.

4.2 Targeting rosacea

The mechanisms we selected that are expected to underlie rosacea are:

- Overexpression of NFκB
- Increased neutrophil activity
- Increased activity of *Staphylococcus epidermidis* and TLR2 on the skin
- Increased pH

Interaction between different mechanisms

In rosacea patients *S. epidermidis* may activate TLR2 receptors (Hajjar *et al.,* 2001) on keratinocytes which eventually results in the production of inflammatory cytokines and chemokines (via NFkB)

(Medzhitov, 2001). The NFκB pathway could be targeted to reduce the symptoms in rosacea. The chemokines attract neutrophils to the site of keratinocyte activation. Neutrophils are more active in rosacea patients when the patients are genetically predisposed. They have TLR2 receptors that, when activated, initiate the production of cathelicidins, nitric oxide and ROS (Nathan, 2006). In rosacea patients these three substances have elevated levels compared to healthy individuals (Peus *et al.,* 1999; Yamasaki *et al.,* 2007; McAleer and Powell, 2010). Neutrophil (activity) reduction could be a target to reduce the symptoms in rosacea. Rosacea patients have more active *S. epidermidis* on their skin (Dahl *et al.,* 2004) and therefore more TLR2 receptors are activated (Hajjar *et al.,* 2001). If in some way *S. epidermidis* is inhibited or if the bacterium has competition, the symptoms of rosacea might be less severe. In one study it was found that formation of a biofilm of the yeast *Candida albicans* promoted the growth of *S. epidermidis* (El-Azizi *et al.,* 2004). *C. albicans* on the skin can be targeted to reduce the amount of *S. epidermidis*. These mechanisms are all connected with each other. A few other bacteria, like *B. oleronius* and *H. pylori,* are suspected to cause disease in rosacea patients. Competition for nutrition or space with other commensal skin bacteria could reduce the symptoms in rosacea.

4.2.1 Overexpression of NFκB

Yersinia enterocolitica is a bacterium that induces IL-10 production in macrophages and inhibits NFκB with YopP (Yersinia outer protein P). IL-10 suppresses pro-inflammatory cytokine production by other immune cells (Hornef *et al.,* 2002). This bacterium species contains many strains which are pathogenic to humans (Bottone, 1997). Furthermore, since most of the strains reside in the gut, the bacterium itself might not be able to survive on the skin. These two factors indicate that the bacterium *Y.enterocolita* is not suitable for application on the skin. However, the YopP protein could be used as a postbiotic in a cream or spray to target the inflammation in rosacea. The skin in rosacea patients is probably inflamed due to the activation of the NFκB pathway (Medzhitov, 2001) and this could be inhibited by the YopP protein.

4.2.2 Increased ROS levels

Salmonella is a bacteria with some alterations in the LPS structure which decreases the innate immune response of the host. In a study by Eriksson *et al.* (2000) several mutants of *S. typhimurium* were tested for the downregulation of nitric oxide expression in macrophage-like cells. They found that *S. typhimurium* secretes effector proteins that interfere with iNOS and therefore the expression of nitric oxide inside immune cells. Furthermore, *S. typhimurium,* like many other bacteria, is able to detoxify ROS enzymatically (Hornef *et al.,* 2002). Elevated ROS (Peus *et al.,* 1999) and nitric oxide (McAleer and Powell, 2010) levels are associated with rosacea and therefore *S. typhimurium* could reduce rosacea symptoms. The bacterium *S. typhimurium* is pathogenic, so the bacterium itself cannot be used for application on the skin (Altekruse *et al.*, 1997) However, the bacterial products could be a possible postbiotic for rosacea. Since the specific effector proteins of *S. typhimurium* are not known, this needs to be investigated first in order to produce a safe product.

4.2.3 Increased neutrophil activity

Mycobacteria can induce the production of anti-inflammatory cytokines. When macrophages are infected by pathogenic *Mycobacteria* they produce IL-6, TGFβ and IL-10. IL-10 inhibits ROS production and suppresses pro-inflammatory cytokine production by other immune cells (Hornef *et al.,* 2002). TGFβ inhibits the activation of some T-cells. Since T-cells stimulate neutrophils by secreting

TNF, TGFβ indirectly inhibits neutrophils (Abbas *et al.,* 2012). Macrophages normally produce less TNF when invaded by *Mycobacteria*. This reduces the activity of neutrophils (Abbas *et al.,* 2012). The altered interleukin secretion may induce regulatory T-cells, which down regulate the immune system by the down regulation of cytokines e.g. IFNγ via TGFβ (Hornef *et al.,* 2002). *M. smegmatis* and *M. phlei,* both members of the phylum *Mycobacteria,* are non-pathogenic bacteria that can inhabit the skin surface (Beltan *et al.,* 2000). When these strains have the same beneficial properties of the pathogenic *Mycobacteria,* they are promising for further research. In rosacea, the skin is inflamed and this can be inhibited by *Mycobacteria.* Therefore these bacteria could work as probiotic.

4.2.4 Increased activity of Staphylococcus epidermidis and TLR2 on the skin

The activity of *S. epidermidis* is higher in the skin of rosacea patients due to a higher skin temperature compared to controls (Dahl *et al.,* 2004). The phenol-soluble modulin is a factor that is secreted by *S. epidermidis* and acts as a ligand for TLR2 (Hajjar *et al.,* 2001). Since the TLR2 receptor is over activated in the skin of rosacea patients, it is important to reduce the number of *S. epidermidis* on the skin*. Pseudomonas aeruginosa* is a commensal skin bacterium that produces several antibiotics. In a healthy host, commensal skin bacteria do not cause disease. *P. aeruginosa* inhibits the growth of the yeast *Candida* (Kerr, 1994) which promotes the growth of *S. epidermidis.* Therefore *P. aeruginosa* can indirectly inhibit the growth of *S. epidermidis* (El-Azizi *et al.,* 2004)*. Pseudomonas* species produce and secrete an antibiotic called pseudomonic acid A (also called mupirocin). *S. epidermidis,* several other staphylococci and streptococci are susceptible against this antibiotic (Sutherland *et al.,* 1985). *P. aeruginosa* is a gram negative bacterium (Beveridge, 1999) this causes it to activate TLR4 instead of TLR2. *P. aeruginosa* could be a potent probiotic to reduce the symptoms in rosacea patients by inhibiting the growth of *S. epidermis*. However, *P. aeruginosa* can act as an opportunistic pathogen and cause skin infections in a weakened host (Andonova and Urumova, 2013). Therefore, the use of this bacterium should be done with caution.

Corynebacteria are mostly commensal skin bacteria that grow in moist areas. They inhabit a large part of the face (Grice and Segre, 2011). However, some of the *Corynebacterium* species are pathogenic (Guarino *et al.,* 1987; Lee *et al.,* 2005). *C. xerosis, C. minutissimum,* and *C. striatus* are non-pathogenic bacteria that grow in areas rich in lipids and sebum; hence they like to colonize the facial skin. They do not produce toxins and are not motile (Chiller *et al.,* 2001). These *Corynebacteria* are gram-positive and have a peptidoglycan layer that could be recognized by TLR2 and make rosacea worse. However, they also have a top layer with free polysaccharides, glycolipids and proteins which are not recognized by TLR2 (Burkovski, 2013) and therefore do not initiate the production of pro-inflammatory cytokines. These *Corynebacteria* could compete with *S. epidermidis* and activate less TLR2 receptors. Therefore, these bacteria could be suitable for topical treatment of rosacea.

4.2.5 Increased pH

Lactobacilli are lactic acid bacteria that can be recognized by TLR2 because of the lipoteichoic acid that is present on the cell wall (Matsuguchi *et al.,* 2003). The lactic acid that *Lactobacilli* produce is an acidic substance and could decrease the pH of the skin. In rosacea patients the pH is increased (Raghallaigh and Powell, 2009; as cited in Lacey and Powell, 2010). Therefore, *Lactobacilli* may be used in the treatment of rosacea. However, *Lactobacilli* could induce a strong TLR2 response. Therefore, only the lactic acid could be used as a postbiotic to decrease the pH of the skin. In this way the skin barrier can improve and the host enzymes will work better for combatting pathogens. Other lactic acid producing bacteria could also be used to reduce symptoms in rosacea.

4.2.6 Summary and recommendation rosacea

In summary, mechanisms potentially underlying rosacea that could be targeted include the overactivity of TLR2 receptors, increased neutrophil activity, increased abundance of *S. epidermidis* and increased pH. No bacteria we found were particularly promising for rosacea. We think that the most promising way to target rosacea using probiotics would be by using the bacteria found as normal commensal residents on the skin. These would have the highest chances of survival on the skin, as well as likely being safe to apply. The bacteria meeting this criterion for rosacea are *M. smegmatis, M. phlei, Corynebacteria* and *Pseudomonas aeruginosa*. However, based on the literature available at this moment, we would not yet recommend treating rosacea using probiotics. For *Corynebacteria*, more information is needed on which specific strains might be beneficial, while for *Pseudomonas aeruginosa* more information is needed on substrains that are safe and unlikely to act as opportunistic pathogens.

An alternative for topical probiotics could be the use of oral probiotics. Oral probiotics can influence the 'gut-brain-skin' axis. Probiotics and their secreted substances can interact with the lymphoid tissue in the gut which is almost 70% of the whole immune system (Bowe, 2013). Oral probiotics can regulate the release of cytokines by inducing regulatory T-cells and effector T-cells (Hacini-Rachinel *et al.,* 2009). Furthermore, when oral probiotics eradicate the bacterial overgrowth in the intestines of rosacea patients, the skin improves too (Parodi *et al.,* 2008 as cited in Lazaridou et al, 2011; Whitehead, 2009). Therefore, for some rosacea patients an oral (probiotic) treatment may be best in the near future. This shows that the intestine and skin are interconnected.

4.3 Targeting acne vulgaris

Mechanisms underlying acne that we selected to target:

- Inflammation
- *Propionibacterium acnes* overgrowth

4.3.1 Inflammation

We found two bacterial strains that could potentially suppress inflammation in acne by counteracting pro-inflammatory factors. Mikelsaar and Zilmer (2009) describe a probiotic strain that showed both antimicrobial and antioxidative effects when fed to mice, namely *Lactobacillus fermentum* ME-3 DSM-14241. These properties may be useful when taken orally. Unfortunately no research could be found for testing topical application of this probiotic.

As described previously, substance P may be involved in the pathogenesis of acne, especially in the induction of inflammation (Lee et al. 2008). An *in vitro* and *ex vivo* study by Guéniche *et al.* (2010) investigated the reactive skin and used substance P to induce inflammation *ex vivo* in a human abdominal plastic skin explant. Their results indicated that *Lactobacillus paracasei* CNCM I-2116 (ST11) lysate was able to reduce TNFα release, vasodilation, oedema and mast cell degranulation which were increased by substance P compared to control. It is possible that ST11 has the same effect in skin cells, although further research is needed to confirm this (Guéniche *et al.*, 2010).

4.3.2 Propionibacterium acnes overgrowth

The overgrowth of *P. acnes* has been associated with the development of inflammation in acne. We found several bacterial strains that are capable of inhibiting *P. acnes*.

Kang *et al.* (2009) isolated a lactic acid bacterium from a human faecal sample, namely *Enterococcus faecalis* SL-5, that showed a particularly high antimicrobial activity against *P. acnes*. This effect of *E. faecalis* SL-5 was mainly due to its secretion of the bacteriocin ESL5. A clinical trial in which patients with mild to moderate acne applied a lotion containing concentrated ESL5 for 8 weeks showed a significant reduction in inflammatory lesions in these patients (Kang *et al.*, 2009). Similarly, Bowe *et al.* (2006) found that 5 of 33 *Streptococcus salivarius* strains they isolated from tongue were able to inhibit *P. acnes*. Wang *et al.* (2014) co-cultured skin microorganisms from human fingerprints with *P. acnes* (ATCC6919) on agar plates under anaerobic conditions. The researchers identified several colonies of skin microorganisms that were able to inhibit the growth of *P. acnes*. The 16S rRNA of 9 of these colonies was analysed to determine the species responsible for this inhibition. One of the 16S rRNA genes shared 96% homology with the 16S RNA of *Paenibacillus* sp. Y412MC10, whereas the other 8 shared 97-99% homology with *S. epidermidis* ATCC12228 or RP62A (Wang *et al.*, 2014). Interestingly, this inhibitory effect was only seen in the presence of glycerol. This suggests that *S. epidermidis* may mainly inhibit *P. acnes* via glycerol fermentation but not by antibiotic protein or peptide. Therefore, the researchers also checked the glycerol fermentation products of the inhibiting skin microorganisms. Four SCFAs were detected, namely acetic acid, butyric acid, lactic acid and succinic acid. From these, succinic acid had the strongest inhibitory effect on *P. acnes* survival. Succinic acid can passively diffuse through the cell wall of microorganisms, thereby decreasing intracellular pH and killing the bacteria. The researchers suggest that live *S. epidermidis* can potentially be used as a bacteriotherapy in acne.

4.3.3 Summary and recommendation acne

Mechanisms underlying acne that we selected as the most promising to target are inflammation and the overabundance of *P. acnes.* Little research has been done on bacteria that could potentially reduce the inflammation observed in acne. In our view, a more promising approach would be the use of topical probiotics inhibiting *P. acnes*. Several strains capable of doing this have been identified, the most promising of which is *S. epidermidis*. This commensal bacterium is a normal resident on the skin, and therefore is likely safe. It also showed clear inhibitory effects on *P. acnes*. However, most studies were performed *in vitro*, or when clinical studies were performed, the bacterial lysate (lysed cells, so dead bacteria) was used. More research is needed on which specific substrains may be the most suitable and their efficacy in humans should be assessed in clinical trials.

4.4 Targeting atopic dermatitis

The mechanisms involved in AD that we selected for targeting:

- *Staphylococcus aureus* infection
- Decreased ceramide levels
- Decreased immune tolerance

4.4.1 Staphylococcus aureus infection

Staphylococcus aureus infection can be targeted in two ways: firstly, by inhibiting *S. aureus* using *S. epidermidis* or other bacterial strains and secondly by increasing the activity of host defensins.

Several studies have investigated the ability of certain strains of *Staphylococcus epidermidis* to inhibit colonization by the pathogen *S. aureus*. Competition between different bacteria in the nose had been established previously, indicating a negative correlation between *S. aureus* colonization and *S.*

epidermidis as well as *Corynebacterium* spp. (Lina *et al.*, 2003). Most research on this topic has focused on the nasal cavity, as this is the main reservoir for *S. aureus*; the dominant commensal bacterium there is *S. epidermidis*. The inhibitory effect of some *S. epidermidis* strains is likely due to the serine protease Esp, which can only be secreted by a subset of *S. epidermidis* (Iwase *et al.*, 2010). Iwase *et al.* (2010) found that the presence of Esp-secreting *S. epidermidis* in human volunteers correlated with the absence of *S. aureus.* Purified Esp inhibits *S. aureus* biofilm formation, destroys pre-existing *S. aureus* biofilms and increases the susceptibility of *S. aureus* in biofilms to immune system components (Iwase *et al.,* 2010). In human volunteers carrying *S. aureus*, purified Esp as well as Esp-secreting *S. epidermidis* eliminated *S. aureus* colonization (Iwase *et al.*, 2010). As to the mechanisms of action of Esp, it has proteolytic activity and degrades specific proteins involved in biofilm formation and proteins associated with colonization. Furthermore, Esp selectively degraded several proteins from the host, such as extracellular matrix (ECM) proteins, which serve as attachment sites for *S. aureus* colonization and infection (Sugimoto *et al.*, 2013). Another study found that Esp cleaves autolysin and thereby prevents the release of *S. aureus* DNA, which works as extracellular matrix in biofilms (Chen *et al.*, 2013).

Subsequent studies have found contradicting results considering Esp and inhibition of *S. aureus*. On the one hand, Park *et al.* (2011) found that mice pre-colonized with Esp-secreting *S. epidermidis* were more resistant to colonization by methicillin-resistant *Staphylococcus aureus* (MRSA). On the other hand, a study by Fredheim *et al.* (2014) found no correlation between Esp expression and *S. aureus* biofilm inhibitory activity. It should be noted that it is unknown whether Esp has an effect on other microbes.

Some other bacterial strains that may inhibit *S. aureus* were investigated in a study by Prince *et al.* (2012). They investigated the effects of three probiotic bacterial strains, namely *Lactobacillus reuteri* ATCC 55730, *L. rhamnosus* AC413, and *L. salivarius* UCC118, on inhibition of *Staphylococcus aureus*. Using an *in vitro* primary human keratinocyte model, they showed that *L. reuteri* and *L. rhamnosus* could significantly inhibit *S. aureus*-induced keratinocyte cell death, with *L. reuteri* showed the most pronounced effect. Notably, this was only the case when the probiotics were applied before or simultaneously with infection with *S. aureus*, but not after infection had started. *L. reuteri* inhibited *S. aureus* by competitive exclusion, not by directly inhibiting growth of *S. aureus*.

Besides its inhibitory effect on *S. aureus*, Esp also greatly potentiates the effect of the antimicrobial host protein hBD2 towards *S. aureus* in biofilms (Iwase *et al.,* 2010). Furthermore, *S. epidermidis* also produces another small molecule that influences host defensins. This molecule increases mRNA expression of the antimicrobial peptides human β-defensin-2 (hBD2) and hBD3. This increased inhibition of *S. aureus* and group A *Streptococcus* growth in cultured undifferentiated human keratinocytes (Lai *et al.*, 2010). *S. epidermidis* cultured media also decreased susceptibility to infection by group A *Streptococcus* in mice. Activation of TLR2 was shown to be crucial to these processes (Lai *et al.*, 2010).

4.4.2 Decreased ceramide levels

As mentioned earlier, ceramide metabolism is altered in AD (Meckfessel and Brandt, 2014). This results in impaired skin barrier function. As explained in the part about Targeting healthy skin, *Streptococcus thermophilus* is able to produce sphingomyelinase, thereby increasing ceramide levels and improving skin barrier function. One study in AD patients showed that application of a cream

containing sonicated (lysated) *Streptococcus thermophilus* for 2 weeks led to a significant increase in skin ceramide levels and improvement of AD symptoms (Di Marzio, 2003).

4.4.3 Decreased immune tolerance

AD patients react stronger to allergens than healthy individuals and thus have decreased immune tolerance (Leung and Bieber, 2003). To reduce the sensitivity of the immune system *Vitreoscilla filiformis* could be helpful.

One mechanism proposed to account for the effects of *V. filiformis* in AD is the induction of manganese superoxide dismutase (MnSOD or SOD2) mRNA expression. MnSOD is an enzyme that is important in the protection against ROS (Mahé *et al.*, 2006). Interestingly, the authors of this study also showed that *V. filiformis* extract could protect against sunburn (Mahé *et al.*, 2006). Another mechanism thought to contribute to the effects of *V. filiformis* lysate is the induction of IL-10-producing dendritic cells and regulatory T-cells. IL-10 is an anti-inflammatory cytokine and regulatory T-cells are important in immune tolerance (Volz *et al.*, 2014). Thus, *V. filiformis* seems to have an immunomodulatory effect that leads to reduced severity of AD symptoms. Another study showed that *V. filiformis* extract combined with LRP spa water may induce the TLR2/PKCz pathway (Mahé *et al.*, 2013).

Several clinical studies have investigated the effect of *V. filiformis* on atopic dermatitis. *Vitreoscilla filiformis* is a filamentous bacterium that is found in sodium-rich spa waters (Guéniche *et al.*, 2008a). The first published study tested an ointment containing 5% *V. filiformis* extract on thirteen patients in a randomised, double-blind, vehicle-controlled trial (Guéniche *et al.*, 2006). Patients applied the treatment and vehicle (ointment without *V. filiformis*) on symmetrical AD lesions (left vs. right side of body) twice a day for 4 weeks. After 28 days of treatment, AD lesion symptoms were significantly improved at the side treated with *V. filiformis* extract compared to vehicle, as measured using the modified eczema area severity index (mEASI). The mild side effects reported were most likely caused by the vehicle and not by the bacterial lysate (Guéniche *et al.*, 2006). The same research group also tested a combination of *V. filiformis* with La Roche Posay (LRP) spa water, which has long been used to treat inflammatory skin disorders. They conducted a mono-centre study with ten participants, in which symmetrical AD lesions were treated with a cream containing 5% LRP with *V. filiformis* or control (vehicle with LRP water but no *V. filiformis*) twice daily for 4 weeks. The cream containing *V. filiformis* improved the symptoms to a greater extent than the control (Guéniche *et al*., 2008a).

Another study by Guéniche *et al.* (2008b) was a prospective, randomized, double-blind, placebo-controlled clinical trial with 75 participants who had mild AD. Subjects received either cream with 5% *V. filiformis* lysate or vehicle and applied this daily for 30 days. The researchers found that *V. filiformis* lysate decreased SCORAD levels, pruritus and sleep loss compared to control. Furthermore, in patients colonized with *S. aureus*, colonization by this pathogen decreased. Transepidermal water loss (a measure for skin barrier function) was not different between cream containing *V. filiformis* lysate and vehicle (Guéniche *et al.*, 2008b). A paper commenting on this study however, posed that the statistical analysis performed in this clinical trial may not have been sufficient, especially relating to sample size calculation (La Colla *et al.*, 2009).

4.4.4 Summary and recommendation AD

We selected *Staphylococcus aureus* infection and decreased ceramide levels as the most promising mechanisms to target in AD. Furthermore, we found a series of promising clinical studies investigating the effect of *V. filiformis* lysate on AD. The best researched strategy for improving AD is treatment with *V. filiformis* lysate. However, the clinical trials were performed with heat-killed bacteria. As the effect of living bacteria may be different, studies should be performed testing the effects of this bacterial strain while alive. Notably, the majority of these studies were performed by a research group that is part of L'Oréal. Promising alternatives are inhibition of *S. aureus* using Esp-secreting *S. epidermidis* strains and improvement of skin barrier function using *Streptococcus thermophilus*. Keep in mind that it is unknown whether Esp affects other microorganisms.

4.5 Market study

The market study resulted from the internet search showed that the majority of probiotics products are for prevention, healthy skin and skin cleaning while very few of them are for the treatment of a skin disease. The detailed mechanisms are not available on the Internet, however, certain mechanisms are mentioned which indicate the way the products work. The main mechanisms are: bacterial displacement (Magoroku Skin Lotion, products from the Unstress line and the cleaning products from Chrisal) and strengthening of the skin with the help of probiotics (products from the Unstress line and the products from Tensall Bio-tech). This indicates that these mechanisms could be used in the treatment of a disease.

If the allergen removing spray really works it may be a possible to test this for human use. In allergic mediated diseases, like atopic dermatitis the prevention of allergens to reach the immune system could greatly decrease the skin's inflammatory reaction.

The cleaning products of Chrisal show that the issue of packaging of live bacteria can be solved via the spore forming mechanism. Spore formation could therefore be a very important factor in selecting the strain because keeping the probiotics alive in a product for an extended amount of time is a significant challenge in designing the product. Magoroku skin lotion uses a different mechanism to package the probiotics. Through fermentation processes, in which a specific temperature is used for each component, the final product is stable. They claim that this process ensures that there is no need for special storage conditions. Another way to circumvent the shelf life problem is to use prebiotics (Nude Miracle mask) or postbiotics (TS7©, RADIII©, HOFF© and Sesative©). Since pre- and postbiotics are not alive it is less difficult to store the product for a longer time.

The possible presence of allergens in the product is an important aspect to think about. Some of them, such as milk or soy, cause trouble for certain people. However, certain products circumvent this problem by fermentation such as Magoroku skin lotion. The long fermentation process causes allergy-causing proteins to be broken down into a substance that is no longer recognized by the immune system as allergic (Magoroku Skin Lotion). This indicates that allergenic substances can be used in long-term fermentation processes which pre-digest and structurally modify the allergic proteins to eliminate an allergic reaction.

The absence of skin care products containing probiotics in stores indicates that this is a rather new concept with great market opportunities. However, we cannot conclude anything yet since the

sample size was very small: the amount of stores available in Wageningen is limited, so a more wide study is needed.

One remark on these results is the fact that the bacteria used for the skin products are mostly gut probiotic bacteria, like *Lactobacillus* and *E. faecalis*. Therefore, these bacteria might not survive on the skin because of the different environment and exert no effect.

4.6 Overall discussion

For healthy skin and atopic dermatitis research has already been done using bacterial lysate in clinical trials. For acne only *in vitro* studies have been performed with potential probiotic strains. For rosacea no promising probiotics were found yet. More research needs to be done to find topical probiotic treatments for rosacea. However, for some rosacea patients an oral probiotic treatment may be best in the near future.

In our research, we mainly focused on bacteria that are known to occur on human skin, as the skin is quite a harsh environment for microorganisms to survive (Krutmann, 2009). Bacteria that normally occur on the skin are likely to survive on it and potentially colonize the skin.

All of the clinical trials we found used bacterial lysate or heat-killed bacteria. These could have profoundly different effects than living bacteria. However, bacterial lysate or heat-killed bacteria might be more convenient to process into a topically applied product than living bacteria, as the product would probably be more stable and there is no risk of pathogenicity. Products found in the market study also often used bacterial lysate, although some contained bacterial spores, which also remain stable over time.

An important consideration in the development of probiotic products is their storage. The probiotics need to remain viable and stable during storage. When stored the product should also remain free of contamination.

The combination of probiotics with prebiotics could be an effective way to make sure the probiotics survive longer and thus increase the chances of their colonization. However, if you would put live probiotics together with prebiotics, the probiotics would already consume the prebiotics before the product is used. Therefore, it may be a good idea to use a two-component system, in which they are combined just before application. This same system could also be used when using freeze-dried bacteria (de Valdez *et al.*, 1985), so liquid is added and bacteria are activated only immediately before application. Using freeze-dried bacteria would ensure that the population remains stable. Another way to store probiotics is to use spores from spore-forming bacteria. These spores can survive in a hostile environment for a prolonged period of time, making it suitable for long-term storage.

Combining pro- and postbiotics could be useful for example if you want to combine the effects of multiple bacteria, but one is potentially pathogenic. In this case, you could use the (safe) postbiotics produced by that bacterium and add it to the probiotics. Another idea to increase the lifespan of the bacteria is to encapsulate them in vesicles (Cook *et al.*, 2012; Govender *et al.*, 2014).

Other things that need to be kept in mind in the development of probiotics containing products for the skin are safety of selected probiotic strain and European legislation.

5. CONCLUSIONS AND ADVICE

Healthy skin

Healthy skin could be improved using sphingomyelinase-producing *Streptococcus thermophilus*. Sphingomyelinase catalyses the hydrolysis of sphingomyelins into ceramides. Ceramides are important components of the *stratum corneum* and are crucial in maintaining skin barrier function. Therefore, increasing the amount of ceramides by increasing the amount of sphingomyelinase could improve skin barrier function. Lysated *S. thermophilus* has already been shown to increase ceramide levels in the *stratum corneum* of the skin. *S. thermophilus* is a safe probiotic strain that is commonly used in oral probiotic formulations such as VSL#3. As regulations for the use of bacteria as oral probiotics are very strict and the strain is safe for consumption, it is probably also safe for use on the skin. **We advise Skinwiser to test living *S. thermophilus* bacteria that produce adequate amounts of sphingomyelinase on healthy skin of volunteers.**

Rosacea

No bacterial strains were found that were promising for topical treatment of rosacea. The only bacterial species we found that might have a beneficial effect on rosacea was *Pseudomonas auruginosa*, but we do not think this is a safe choice. As rosacea is more prevalent in lighter skin and UV radiation contributes to development of inflammatory lesions in rosacea, protection against sun damage may improve rosacea. During the market study, we found that the Unstress line from the Christina Company claims to relieve stressed skin resulting from amongst others sun exposure. This product line contains *Lactobacilli*. The company claims to have performed research and that their products are clinically proven, although no references to scientific articles were found. Furthermore, we found studies indicating that oral probiotics can improve protection against and recovery from sun damage (see section *3.1.3 Photoprotection by oral probiotics*). The bacterial strains used in these studies were *Lactobacillus johnsonii* NCC 533 and *L. plantarum* HY7714. Thus, these *Lactobacillus* strains could improve rosacea symptoms when used orally. *Lactobacilli* may also have a beneficial effect through their production of lactic acid, thereby increasing the pH of the skin.

Furthermore, rosacea patients have a higher prevalence of SIBO. When rosacea patients were treated for SIBO, their rosacea symptoms decreased. A promising way to treat SIBO could be by using oral probiotics. Thus, the use of oral probiotics in rosacea patients with SIBO could improve their rosacea symptoms. Literature study was not performed to find specific strains that could be used for this.

Another possibility for treating rosacea is targeting IAPs, which are enzymes that suppress the production of pro-inflammatory cytokines upon LPS recognition. For example, oral administration of IAPs can reduce rosacea symptoms. Reduced amounts or activity of IAPs may underlie rosacea, as discussed in section *3.2.3 Risk factors*. Additionally, a potential target that Skinwiser could look further into is *Demodex* mites, because they may be more prevalent in rosacea patients. Lastly, Skinwiser could look into the diet of rosacea patients, since certain foods seem to worsen rosacea symptoms while others seem to relieve them.

We advise Skinwiser not to test topical probiotics to improve rosacea. Instead, we advise Skinwiser to focus on oral probiotics for rosacea.

Acne

Acne could be improved using certain *Staphylococcus epidermidis* substrains to inhibit *Propionibacterium acnes*. *P. acnes* overgrowth has been associated with the development of inflammation in acne. Three strains capable of inhibiting *P. acnes* are *Enterococcus faecalis* SL-5, *Streptococcus salivarius* and *Staphylococcus epidermidis*. From these, *S. epidermidis* is most likely to survive on the skin, as it is a common skin commensal. IL-8 plays an important role in inflammation in acne. The probiotic *Lactobacillus salivarius* was shown to inhibit IL-8 release when taken orally by mice inoculated with *Helicobacter pylori*. The microbiome of these mice was controlled by the researchers. However, *L. salivarius* is unlikely to survive long on the skin. **We advise Skinwiser to ascertain the safety of *P. acnes*-inhibiting *S. epidermidis* and if it is safe, to test topical application of *S. epidermidis* on the skin of acne patients.**

Atopic dermatitis

Atopic dermatitis could be improved in several ways. During the literature study, we found several promising bacterial strains. First of all, *Vitreoscilla filiformis* can be used to improve AD symptoms. This bacterium likely works by increasing ROS protection, reducing inflammation and improving immune tolerance. Several clinical studies using *V. filiformis* lysate have already shown the therapeutic potential of this bacterium. No information could be found on the safety of these bacteria. **If it is safe, we advise Skinwiser to test topical application of living *V. filiformis* in patients with atopic dermatitis.**

Secondly, sphingomyelinase-producing *Streptococcus thermophilus* can be used to improve atopic dermatitis. The skin barrier is often disrupted in AD patients. As described previously, sphingomyelinase-producing *Streptococcus thermophilus* could improve the skin barrier by increasing the amount of ceramides in the *stratum corneum*. **We advise Skinwiser to test living *S. thermophilus* bacteria that produce adequate amounts of sphingomyelinase on skin of patients with atopic dermatitis.**

Thirdly, Esp-secreting *S. epidermidis* can be used to improve atopic dermatitis by combatting *S. aureus* infection. *S. aureus* is found in 90% of AD lesions and worsens the symptoms of AD. Esp secreted by *S. epidermidis* can inhibit *S. aureus* and potentiate the effects of hBD2 produced by the host. Keep in mind that the effect of Esp on other microorganisms should be tested before proceeding with clinical studies. Another molecule secreted by *S. epidermidis* increases hBD2 and hBD3 expression. Thus, *S. epidermidis* inhibits *S. aureus* in multiple ways. As *S. epidermidis* is a commensal bacterium normally residing on the skin, it is likely safe for application on the skin. **We advise Skinwiser to ascertain the safety of Esp-secreting *S. epidermidis* and if it is safe, to test topical application of Esp-secreting *S. epidermidis* on the skin of atopic dermatitis patients.**

During the market study, we found one probiotics-containing skin care line aimed at preventing atopic dermatitis, namely RAD-III from Tensall bio-tech. This product line contained *Lactobacillus paracasei*, *L. casei* and *L. rhamnosus* GG. The mechanisms by which these bacteria are supposed to work were not found. It is claimed to work by moisturizing sensitive skin and relieving skin itching, as well as providing skin protection and repair. Thus, Skinwiser could also test these probiotics on the skin of AD patients. However, the bacteria found in the literature study may be more promising, as *Lactobacilli* are unlikely to survive long on the skin.

Another option for treating atopic dermatitis is using oral probiotics. We came across many studies investigating the use of oral probiotics in atopic dermatitis. However, our focus was on topical probiotics so we did not further investigate oral probiotics.

Remark

An important consideration when performing clinical studies is the safety of the recommended commensal bacteria. They may be common residents in the skin microbiome of healthy individuals, but the virulence of the bacteria may be influenced by the health state of the host. For example, the commensal *S. epidermidis* can act as an opportunistic pathogen in vulnerable people. Another important consideration when using bacteria is that the effects of bacterial species can differ from strain to strain. Thus, the efficacy of the strain needs to be verified to make sure the bacteria have the desired effect.

General conclusion

We believe that probiotics could be a promising way to maintain or improve skin health. So far, very little research has been done on the use of probiotics on the skin. For some skin conditions like rosacea it is still too early to target their symptoms using probiotics, but the use of topical probiotics is promising for the improvement of healthy skin, acne and atopic dermatitis.

6. RECOMMENDATION FOR FURTHER RESEARCH

For the next step process, we recommend four bacterial strains for further research before development of skin care products. In *Table 13* the recommended strains, their target, mechanisms, assumed safety, test level and level of testing on the skin are depicted. It is important to keep in mind that different substrains of a species could have different properties. For example, some substrains of *S. epidermidis* secrete Esp, while others do not.

Table 13. Overview of the recommended bacterial strains, their target condition, mechanism of action, assumed safety, test level and if relevant, how it was tested on the skin.

Probiotics	Target	Mechanism	Safety	Test level	Test on skin
S. thermophilus	Healthy skin	Improvement skin barrier by increasing ceramides	Generally recognized as safe	Clinical test	Cream containing sonicated (lysated) bacteria
	Atopic dermatitis	Improvement skin barrier by increasing ceramides	Generally recognized as safe	Clinical test	Cream containing sonicated (lysated) bacteria
S. epidermidis	Acne	Competition with *P. acnes*	Skin flora, certain strains are safe	*In vitro* tests	-
V. filiformis	Atopic dermatitis	Assumed via: - Induction of ROS protection - Anti-inflammatory	Unknown, but commonly occurs in sodium-rich spa water, so might be safe?	Clinical tests	Creams containing bacterial lysate
Esp-secreting *S. epidermidis*	Atopic dermatitis	Competitive exclusion of *S. aureus*	Skin flora, certain strains are safe	Clinical test, only in nasal cavity	Purified Esp and (living) Esp-secreting *S. epidermidis*

Several aspects need to be tested for the different bacteria. *S. thermophilus* and *V. filiformis* have been tested in clinical trials, but only as lysates. As no tests have been done using living bacteria, safety of the living bacteria should be tested first. To do this, an *ex vivo* test using an organ culture of human skin could be appropriate (Varani *et al.*, 2007). This system has been used previously to establish toxicity of chemicals to the skin.

After this, several doses could be checked in humans, starting at a low dosage. Once the safe doses have been established, clinical tests assessing its efficacy could be performed with greater numbers of participants.

S. epidermidis for treatment of acne has only been found to inhibit *P. acnes in vitro*. For a strain capable of doing this, safety and efficacy could be tested according to the method mentioned previously.

Esp-secreting *S. epidermidis* for treatment of atopic dermatitis could be tested in a similar way as well, but keep in mind that the strain needs to be checked for Esp secretion. This could be done using a Western Blot and antibody staining.

Some rosacea patients suffer from SIBO. For these patients, treatment with oral probiotics containing commensal or mutualistic bacteria from the small intestine may improve their symptoms. Rosacea patients without SIBO might benefit from topical probiotics. Several bacteria were found that might possibly improve rosacea, but little is known about the causes of rosacea, making its targeting very difficult.

Clinical assessment

Skin barrier function in healthy skin and atopic dermatitis trials could be tested by measuring transepidermal water loss (e.g. using TEWA meter). Clinical assessment of acne patients could be done using e.g. the Leeds Acne Grading Technique, which is a widely used grading system based on photographic standards (Alison, 2010). In addition, sebum levels of the skin surface could be measured using sebumetry (e.g. using Sebumeter) and acne lesions and the presence of *P. acnes* could be assessed (e.g. Visiopor). For atopic dermatitis, SCORing AD (SCORAD) is commonly used to evaluate improvement of symptoms (Oranje *et al.*, 2007). In addition, skin hydration could be determined using Corneometer and the structure of the skin and dryness using Visioscan.

Storage

Storage of products that contain probiotics is an important factor. The probiotics need to remain viable and stable and stay free of contamination. We think that the use of prebiotics and probiotics together in a product would increase the likelihood of survival. Since the probiotics would already consume the prebiotics before the product is used, we recommend research into the use of a two-component system. Another option to combine prebiotics and probiotics would be the usage of freeze-dried bacteria in a product. In addition, this would ensure that the population remains stable. If bacteria are used that can form spores, the spores can be used for long-term storage.

ACKNOWLEDGEMENTS

We are using this opportunity to express our gratitude to everyone who supported us throughout the project. First and foremost, we would like to thank Dr Carel Weijers for his coaching and support during this project. Furthermore, we are very grateful to Dr Ir Willemien Lommen, who gave us feedback on the project proposal and report. Lastly, we would like to thank Gerda Wink for her feedback on our project proposal.

REFERENCES

Aas, J., Gessert, C. E., & Bakken, J. S. (2003). Recurrent Clostridium difficile colitis: case series involving 18 patients treated with donor stool administered via a nasogastric tube. *Clinical infectious diseases*, *36*(5), 580-585.

Abad-Casintahan, F., Chow, S. K., Goh, C. L., Kubba, R., Miyachi, Y., Noppakun, N., JoAnn, S. Dae, H. S., Ynag, L. & Kang, S. (2011). Toward evidence-based practice in acne: Consensus of an Asian Working Group. *The Journal of dermatology*, *38*(11), 1041-1048.

Abbas, A.K., Lichtman, A.H., Pillai, S., (2012) *Cellular and molecular immunology*. Philadelphia: Elsevier Saunders.

Abram, K., Silm, H., Maaroos, H. I., & Oona, M. (2010). Risk factors associated with rosacea. *Journal of the European Academy of Dermatology and Venereology*, *24*(5), 565-571.

Akdis, C. A., Akdis, M., Bieber, T., Bindslev-Jensen, C., Boguniewicz, M., Eigenmann, P., Hamid, Q., Kapp, A., Leung, D. Y. M., Lipozencic, J., Luger, T. A., Muraro, A., Novak, N., Platts-Mills, T. A. E., Rosenwasser, L., Scheynius, A., Simons, F. E. R., Spergel, J., Turjanmaa, K., Wahn, U., Weidinger, S., Werfel,T. & Zuberbier, T. (2006). Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Report. Allergy, 61(8), 969-987.

Aktan, F. (2004). iNOS-mediated nitric oxide production and its regulation. *Life sciences*, *75*(6), 639-653.

Al Robaee, A. A. (2005). Prevalence, knowledge, beliefs and psychosocial impact of acne in University students in Central Saudi Arabia. *Saudi medical journal*, *26*(12), 1958-1961.

Aldana, O. L., Holland, D. B., & Cunliffe, W. J. (1998). Variation in pilosebaceous duct keratinocyte proliferation in acne patients. *Dermatology*, *196*(1), 98-99.

Altekruse, S. F., Cohen, M. L., & Swerdlow, D. L. (1997). Emerging foodborne diseases. *Emerging infectious diseases*, *3*, 285-294.

Altrichter, S., Kriehuber, E., Moser, J., Valenta, R., Kopp, T., & Stingl, G. (2008). Serum IgE autoantibodies target keratinocytes in patients with atopic dermatitis. *Journal of Investigative Dermatology*, *128*(9), 2232-2239.

Andonova, M., Urumova, V. (2013). Immune surveillance mechanisms of the skin against the stealth infection strategy of *Pseudomonas aeruginosa* – Review. *Comparative Immunology, Microbiology and Infectious Diseases*, *36(5)*, 433-448.

Angermeier, M. C. (1999). Treatment of facial vascular lesions with intense pulsed light. *Journal of Cosmetic and Laser Therapy*, *1*(2), 95-100.

Anttila, H. S. I., Reitamo, S., & Saurat, J. H. (1992). Interleukin 1 immunoreactivity in sebaceous glands. *British Journal of Dermatology*, *127*(6), 585-588.

Arciola, C. R., Bustanji, Y., Conti, M., Campoccia, D., Baldassarri, L., Samorì, B., & Montanaro, L. (2003). Staphylococcus epidermidis fibronectin binding and its inhibition by heparin. *Biomaterials*, *24*(18), 3013-3019.

Argenziano, G., Donnarumma, G., Arnese, P., Assunta Baldassarre, M., & Baroni, A. (2003). Incidence of anti-Helicobacter pylori and anti-CagA antibodies in rosacea patients. *International journal of dermatology*, *42*(8), 601-604.

Arikawa, J., Ishibashi, M., Kawashima, M., Takagi, Y., Ichikawa, Y. & Imokawa, G. (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)*, 433-439.

Armstrong, D.C. & Johns, M.R. (1997). Culture conditions affect the molecular weight properties of hyaluronic acid produced by *Streptococcus zooepidemicus*. *Applied and Environmental Microbiology*, *63(7)*, 2579-2764.

Banat, I. M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M. G., Fracchia, L., Smyth. T . J & Marchant, R. (2010). Microbial biosurfactants production, applications and future potential. *Applied Microbiology and Biotechnology*, *87*(2), 427-444.

Bates, J. M., Akerlund, J., Mittge, E., & Guillemin, K. (2007). Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. *Cell host & microbe*, *2*(6), 371-382.

Beltan, E., Horgen, L., & Rastogi, N. (2000). Secretion of cytokines by human macrophages upon infection by pathogenic and non-pathogenic mycobacteria. *Microbial pathogenesis*, *28*(5), 313-318.

Beltrani, V. S. (1999). The clinical spectrum of atopic dermatitis. *Journal of allergy and clinical immunology*, *104*(3), S87-S98.

Bender, B. G., Leung, S. B., & Leung, D. Y. (2003). Actigraphy assessment of sleep disturbance in patients with atopic dermatitis: an objective life quality measure. *Journal of allergy and clinical immunology*, *111*(3), 598-602.

Beveridge, T. J. (1999). Structures of gram-negative cell walls and their derived membrane vesicles. *Journal of bacteriology*, *181*(16), 4725-4733.

Bieber, T. (2008). Atopic dermatitis. *The New England Journal of Medicine, 358*, 1483-1494.

Bieber, T., De La Salle, H., Wollenberg, A., Hakimi, J., Chizzonite, R., Ring, J., ... & De la Salle, C. (1992). Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (Fc epsilon RI). *The Journal of experimental medicine*, *175*(5), 1285-1290.

Bikowski, J. B. (2004). Mechanisms of the comedolytic and anti-inflammatory properties of topical retinoids. *Journal of drugs in dermatology: JDD*, *4*(1), 41-47.

Bitar, K., & Reinhold, J. G. (1972). Phytase and alkaline phosphatase activities in intestinal mucosae of rat, chicken, calf, and man. *Biochimica et Biophysica Acta (BBA)-Enzymology*, *268*(2), 442-452.

Boehm, K. D., Yun, J. K., StrohI, K. P., & Elmets, C. A. (1995). Messenger RNAs for the multifunctional cytokines interleukin-1α, interleukin-1β and tumor necrosis factor-α are present in adnexal tissues and in dermis of normal human skin. *Experimental dermatology*, *4*(6), 335-341.

Bos, J. D., & Kapsenberg, M. L. (1986). The skin immune system Its cellular constituents and their interactions. *Immunology Today*, *7*(7), 235-240.

Bottone, E. J. (1997). Yersinia enterocolitica: the charisma continues. *Clinical Microbiology Reviews*, *10*(2), 257-276.

Bouilly-Gauthier, D., Jeannes, C., Maubert, Y., Duteil, L., Queille-Roussel, C., Piccardi, N., Montastier, C., Manissier, P., Piérard, G. & Ortonne, J. (2010). Clinical evidence of benefits of a dietary supplement containing probiotic and carotenoids on ultravoilet-induced skin damage. *British Journal of Dermatology*, *163*, 536-543.

Bowe, W. P. (2013). Probiotics in acne and rosacea. *Cutis; cutaneous medicine for the practitioner*, *92*(1), 6-7.

Bowe, W. P., & Logan, A. C. (2010). Clinical implications of lipid peroxidation in acne vulgaris: old wine in new bottles. *Lipids Health Dis*, *9*, 141.

Bronstein, J. L. (1994). Our current understanding of mutualism. *Quarterly Review of Biology*, 31-51.

Brown, S. K., & Shalita, A. R. (1998). Acne vulgaris. *The Lancet*, *351*(9119), 1871-1876.

Bunikowski, R., Mielke, M., Skarabis, H., Herz, U., Bergmann, R. L., Wahn, U., & Renz, H. (1999). Prevalence and role of serum IgE antibodies to the Staphylococcus aureus–derived superantigens SEA and SEB in children with atopic dermatitis. *Journal of Allergy and Clinical Immunology*, *103*(1), 119-124.

Burkovski, A. (2013). Cell envelope of corynebacteria: structure and influence on pathogenicity. *International Scholarly Research Notices*, *2013*.

Buske-Kirschbaum, A., Gierens, A., Höllig, H., & Hellhammer, D. H. (2002). Stress-induced immunomodulation is altered in patients with atopic dermatitis. *Journal of neuroimmunology*, *129*(1), 161-167.

Callen, J. P., Greer, K. E., Paller, A., & Swinyer, L. (2000). Color Atlas of Dermatology: A Morphological Approach.

Candi, E., Schmidt, R., & Melino, G. (2005). The cornified envelope: a model of cell death in the skin. *Nature reviews Molecular cell biology*, *6*(4), 328-340.

Chamberlain, N. R., & Brueggemann, S. A. (1997). Characterisation and expression of fatty acid modifying enzyme produced by Staphylococcus epidermidis. *Journal of medical microbiology*, *46*(8), 693-697.

Chen, A. C., & Damian, D. L. (2014). Nicotinamide and the skin. *Australasian Journal of Dermatology*.

Chen, C., Krishnan, V., Macon, K., Manne, K., Narayana, S.V.L. & Schneewind, O. (2013). Secreted proteases control autolysin-mediated biofilm growth of *Staphylococcus aureus*. *The Journal of Biological Chemistry*, *288(41)*, 29440-29452.

Chen, Y. C. , Chien Y. W., Chang, P. J., Hsieh, W. S., & Chen, P. C. (2012). Probiotic Supplement Use among Young Children in Taiwan: A Prospective Cohort Study. *PLoS ONE 7(9): e43885.*

Chen, Y.E., Tsao, H. (2013). The skin microbiome: Current perspectives and future challenges. *Journal of the American Academy of Dermatology*, *69(1)*, 143-155.e3.

Cherbut, C., Michel, C., & Lecannu, G. (2003). The prebiotic characteristics of fructooligosaccharides are necessary for reduction of TNBS-induced colitis in rats. *The Journal of nutrition*, *133*(1), 21-27.

Chien, L.J., & Lee, C.K. (2007). Hyaluronic acid production by recombinant *Lactococcus lactis*. *Applied Microbiology and Biotechnology*, *77(2)*, 339-346.

Chiller, K., Selkin, B. A., & Murakawa, G. J. (2001). Skin microflora and bacterial infections of the skin. In *Journal of Investigative Dermatology Symposium Proceedings, 6 (3)*, 170-174). Nature Publishing Group.

Chivot, M. (2005). Retinoid therapy for acne. *American journal of clinical dermatology*, *6*(1), 13-19.

Chosidow, O., & Cribier, B. (2011). Epidemiology of rosacea: updated data. In *Annales de dermatologie et de vénéréologie* (Vol. 138, pp. S179-S183). Elsevier Masson.

Coda, A. B., Hata, T., Miller, J., Audish, D., Kotol, P., Two, A., Shafiq, F., Yamasaki, K., Harper, J. C., Del Rosso, J. Q. & Gallo, R. L. (2013). Cathelicidin, kallikrein 5, and serine protease activity is inhibited during treatment of rosacea with azelaic acid 15% gel. *Journal of the American Academy of Dermatology*, *69*(4), 570-577.

Cogen, A.L., Nizet, V., & Gallo, R.L. (2008) Skin microbiota: a source of disease or defence? *British Journal of Dermatology*, *158*, 442-455.

Cook, M. T., Tzortzis, G., Charalampopoulos, D., & Khutoryanskiy, V. V. (2012). Microencapsulation of probiotics for gastrointestinal delivery. *Journal of Controlled Release*, *162*(1), 56-67.

Cookson, W. O., Ubhi, B., Lawrence, R., Abecasis, G. R., Walley, A. J., Cox, H. E., ... & Harper, J. I. (2001). Genetic linkage of childhood atopic dermatitis to psoriasis susceptibility loci. *Nature genetics*, *27*(4), 372-373.

Costa, C. P. D., Kirschning, C. J., Busch, D., Dürr, S., Jennen, L., Heinzmann, U., Prebeck, S., Wagner, H., & Miethke, T. (2002). Role of chlamydial heat shock protein 60 in the stimulation of innate immune cells by Chlamydia pneumoniae. *European journal of immunology*, *32*(9), 2460-2470.

Crawford, G. H., Pelle, M. T., & James, W. D. (2004). Rosacea: I. Etiology, pathogenesis, and subtype classification. *Journal of the American Academy of Dermatology*, *51*(3), 327-341

Cunliffe, W. J. (1986). Acne and unemployment. *British Journal of Dermatology*, *115*(3), 386-386.

Cunliffe, W. J. (1998). The sebaceous gland and acne–40 years on. *Dermatology*, *196*(1), 9-15.

Cunliffe, W. J., & Gollnick, H. P. (2001). *Acne: Diagnosis and management*. Taylor & Francis.

Cunliffe, W. J., & Shuster, S. (1969). Pathogenesis of acne. *The Lancet*, *293*(7597), 685-687.

Cunliffe, W. J., Holland, D. B., Clark, S. M., & Stables, G. I. (2000). Comedogenesis: some new aetiological, clinical and therapeutic strategies. *British Journal of Dermatology*, *142*(6), 1084-1091.

Dahl, M. V., (2010) ''A Treatment Strategy for Rosacea'' *Pathogenesis and Treatment of Acne and Rosacea*. Ed. Zouboulis, C. C., Katsambas, A. D., & Kligman, A. M. *Springer* 684-690

Dahl, M. V., Ross, A. J., & Schlievert, P. M. (2004). Temperature regulates bacterial protein production: possible role in rosacea. *Journal of the American Academy of Dermatology*, *50*(2), 266-272.

Dawson, A. L., & Dellavalle, R. P. (2013). Acne vulgaris. *BMJ*, *346*(7907), 30-33.

Delzenne, N. M. (2003). Oligosaccharides: state of the art. *Proceedings of the nutrition Society*, *62*(01), 177-182.

Denda, M., Sokabe, T., Fukumi-Tominaga, T., & Tominaga, M. (2006). Effects of skin surface temperature on epidermal permeability barrier homeostasis. *Journal of Investigative Dermatology*, *127*(3), 654-659.

Dessinioti, C., (2010) "Acne Pathogenesis: What We Have Learned Over the Years'' *Pathogenesis and Treatment of Acne and Rosacea*. Ed. Zouboulis, C. C., Katsambas, A. D., & Kligman, A. M. *Springer* 62-67

Di Marzio, L., Centi, C., Cinque, B., Masci, S., Giuliani, M., Arcieri, A., Zicari, L., De Simone, C., Cifone, M.G. (2003). Effect of the lactic acid bacterium *Streptococcus thermophilus* on stratum corneum ceramide levels and signs and symptoms of atopic dermatitis patients. *Experimental Dermatology*, *12*, 615-620.

Di Marzio, L., Cinque, B., De Simone, C., & Cifone, M.G. (1999). Effect of the lactic acid bacterium *Streptococcus thermophilus* on ceramide levels in human keratinocytes *in vitro* and stratum corneum *in vitro*. *Journal of Investigative Dermatology*, *113*, 98-106.

Downing, D. T., Stewart, M. E., Wertz, P. W., & Strauss, J. S. (1986). Essential fatty acids and acne. *Journal of the American Academy of Dermatology*, *14*(2), 221-225.

Downing, D. T., Strauss, J. S., & Pochi, P. E. (1972). Changes in skin surface lipid composition induced by severe caloric restriction in man. *The American journal of clinical nutrition*, *25*(4), 365-367.

Dranoff, G. (2004). Cytokines in cancer pathogenesis and cancer therapy. *Nature Reviews Cancer*, *4*(1), 11-22.

Dréno, B., (2010). "Topical Antibiotics"*Pathogenesis and Treatment of Acne and Rosacea*. Ed. Zouboulis, C. C., Katsambas, A. D., & Kligman, A. M. Springer 628-637

Dubin, G., Chmiel, D., Mak, P., Rakwalska, M., Rzychon, M., & Dubin, A. (2001). Molecular cloning and biochemical characterisation of proteases from Staphylococcus epidermidis. *Biological chemistry*, *382*(11), 1575-1582.

Eady, A. E., Cove, J. H., & Layton, A. M. (2003). Is antibiotic resistance in cutaneous propionibacteria clinically relevant?. *American journal of clinical dermatology*, *4*(12), 813-831.

El-Azizi, M. A., Starks, S. E., & Khardori, N. (2004). Interactions of Candida albicans with other Candida spp. and bacteria in the biofilms*. *Journal of applied microbiology*, *96*(5), 1067-1073.

Elston, D. M. (2009). Topical antibiotics in dermatology: emerging patterns of resistance. *Dermatologic clinics*, *27*(1), 25-31.

Erbagˇci, Z., & Özgöztaşi, O. (1998). The significance of Demodex folliculorum density in rosacea. *International journal of dermatology*, *37*(6), 421-425.

Eriksson, S., Björkman, J., Borg, S., Syk, A., Pettersson, S., Andersson, D. I., & Rhen, M. (2000). Salmonella typhimurium mutants that downregulate phagocyte nitric oxide production. *Cellular microbiology*, *2*(3), 239-250.

Eyre, D.W., Kenkre, J.S., Bowler, I.C.J.W., McBride, S.J. (2010). *Streptococcus equi* subspecies *zooepidemicus* meningitis – a case report and review of the literature. *European Journal of Clinical Microbiology & Infectious Diseases*, *29*, 1459-1463.

FAO/WHO (2002). Guidelines for the Evaluation of Probiotics in Food . Report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food. London, Ontario, Canada.

Farrar, M. D., and Bojar, R. A., (2010) "The Role of Bacteria'' *Pathogenesis and Treatment of Acne and Rosacea.* Ed. Zouboulis, C. C., Katsambas, A. D., & Kligman, A. M. Springer 628-637

Faust, K., Raes, J. (2012). Microbial interactions: from networks to models. *Nature Reviews Microbiology*, *10*, 538-550.

Feldman, S. R., Huang, W. W., & Huynh, T. T. (2014). Current Drug Therapies for Rosacea: A Chronic Vascular and Inflammatory Skin Disease. *Journal of managed care pharmacy: JMCP*, *20*(6), 623-629.

Fimmel, S., Abdel-Naser, M. B., Kutzner, H., Kligman, A. M., & Zouboulis, C. C. (2008). New aspects of the pathogenesis of rosacea. *Drug Discovery Today: Disease Mechanisms*, *5*(1), e103-e111.

Fimmel, S., Glass, E., & Zouboulis, C. C. (2005). Neuropeptides and UV radiation are possible mediators of inflammation in rosacea. *Journal of investigative dermatology*, 124 (4), A16-A16.

Fimmel, S., Kutzner, H., & Zouboulis, C. C. (2010) ''The Vascular Concept'' *Pathogenesis and Treatment of Acne and Rosacea*. Ed. Zouboulis, C. C., Katsambas, A. D., & Kligman, A. M. *Springer* 612-613.

Finegold, S. M., Molitoris, D., Song, Y., Liu, C., Vaisanen, M. L., Bolte, E., McTeague, M., Sandler, R., Wexler, H., Marlowe, E.M., Collins, M.D., Lawson, P.A., Summanen, P., Baysallar, M., Tomzynski, T.J., Reas, E., Johson, E., Rolfe, R., Nasir, P., Shah, H., Haake, D.A., Manning, P. & Kaul, A. (2002). Gastrointestinal microflora studies in late-onset autism. *Clinical Infectious Diseases*, *35*(Supplement 1), S6-S16.

Fitzpatrick, T. B., Johnson, R., Wolff, K., & Suurmond, R. (2005). Melanoma precursors and primary cutaneous melanoma. Fitzpatrick's color atlas and synopsis of clinical dermatology. 5th ed. New York: McGraw Hill, 302-11.

Fleming, A. (1909). On the etiology of acne vulgaris and its treatment by vaccines. *The lancet*, *173*(4467), 1035-1038.

Flohr, C., Pascoe, D., & Williams, H. C. (2005). Atopic dermatitis and the 'hygiene hypothesis': too clean to be true?. *British journal of dermatology*, *152*(2), 202-216.

Fredheim, E.G.A., Flaegstad, T., Askarian, F., Klingenberg, C. (2014). Colonisation and interaction between *S. epidermidis* and *S. aureus* in the nose and throat of healthy volunteers. *European Journal of Clinical Microbiology & Infectious Diseases*, DOI: 10.1007/s10096-014-2197-5.

Gallo, R. L. & Hooper, L. V. (2012). Epithelial antimicrobial defence of the skin and intestine. *Nature Rev. Immunol. 12*, 503-514.

Galobardes, B., Davey Smith, G., Jeffreys, M., & McCarron, P. (2005). Has acne increased? Prevalence of acne history among university students between 1948 and 1968. The Glasgow Alumni Cohort Study. *British Journal of Dermatology*, *152*(4), 824-825.

Gamble, R., Dunn, J., Dawson, A., Petersen, B., McLaughlin, L., Small, A., Kindle, S. & Dellavalle, R. P. (2012). Topical antimicrobial treatment of acne vulgaris. *American journal of clinical dermatology*, *13*(3), 141-152.

Ganceviciene, R., Böhm, M., Fimmel, S., & Zouboulis, C. C. (2009b). The role of neuropeptides in the multifactorial pathogenesis of acne vulgaris. *Dermatoendocrinol*, *1*(3), 170-6.

Georgala, S., Katoulis, A. C., Kylafis, G. D., Koumantaki-Mathioudaki, E., Georgala, C., & Aroni, K. (2001). Increased density of Demodex folliculorum and evidence of delayed hypersensitivity reaction in subjects with papulopustular rosacea. *Journal of the European Academy of Dermatology and Venereology*, *15*(5), 441-444.

George, R., Clarke, S., & Thiboutot, D. (2008, September). Hormonal therapy for acne. In *Seminars in cutaneous medicine and surgery* (Vol. 27, No. 3, pp. 188-196). WB Saunders.

Geppetti, P., Nassini, R., Materazzi, S. and Benemei, S. (2008), The concept of neurogenic inflammation. BJU International, 101: 2–6. doi: 10.1111/j.1464-410X.2008.07493.x

Gibson, G. R. & Roberfroid, M. B. (1995). Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics. *J. Nutr. 125,* 1402-1412.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr Res Rev*, *17*(2), 259-275.

Gil, K. M., Keefe, F. J., Sampson, H. A., McCaskill, C. C., Rodin, J., & Crisson, J. E. (1987). The relation of stress and family environment to atopic dermatitis symptoms in children. *Journal of Psychosomatic Research*, *31*(6), 673-684.

Gleich, G. J. (2000). Mechanisms of eosinophil-associated inflammation. *Journal of Allergy and Clinical Immunology*, *105*(4), 651-663.

Gollnick, H. P. M. (1991). Pathogenesis and pathogenesis related treatment of acne. *J Dermatol*, *18*, 489-499.

Gollnick, H., Cunliffe, W., Berson, D., Dreno, B., Finlay, A., Leyden, J. J., Shalita, A. R., & Thiboutot, D. (2003). Management of acne: a report from a Global Alliance to Improve Outcomes in Acne. *Journal of the American Academy of Dermatology*, *49*(1), S1-S37.

Goulden, V., Stables, G. I., & Cunliffe, W. J. (1999). Prevalence of facial acne in adults. *Journal of the American Academy of Dermatology*, *41*(4), 577-580.

Govender, M., Choonara, Y. E., Kumar, P., du Toit, L. C., van Vuuren, S., and Pillay, V. (2014). A Review of the Advancements in Probiotic Delivery: Conventional vs. Non-conventional Formulations for Intestinal Flora Supplementation. *AAPS PharmSciTech*, *15*(1), 29-43.

Grenham, S., Clarke, G., Cryan, J. F. and Dinan, T. G. (2011). Brain-Gut-Microbe Communication in Health and Disease. *Front. Physiol., 2*(94), 1-16.

Grewe, M., Czech, W., Morita, A., Werfel, T., Klammer, M., Kapp, A. and Krutmann, J. (1998). Human eosinophils produce biologically active IL-12: implications for control of T cell responses. *The Journal of Immunology*, *161*(1), 415-420.

Grice, E. A., & Segre, J. A. (2011). The skin microbiome. *Nature Reviews Microbiology*, *9*(4), 244-253.

Grice, E.A. (2014). The skin microbiome: potential for novel diagnostic and therapeutic approaches to cutaneous disease. *Seminars in Cutaneous Medicine and Surgery*, *33*, 98-103.

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

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

Grice, E.A., Segre, J.A. (2011). The skin microbiome. *Nature Reviews Microbiology*, *9*, 244-253.

Guarino, M. J., Qazi, R., Woll, J. E., & Rubins, J. (1987). Septicemia, rash, and pulmonary infiltrates secondary to Corynebacterium group JK infection. *The American journal of medicine*, *82*(1), 132-134.

Guéniche, A., Bastien, P., Ovigne, J. M., Kermici, M., Courchay, G., Chevalier, V., Breton, L., & Castiel-Higounenc, I. (2010). Bifidobacterium longum lysate, a new ingredient for reactive skin. *Experimental dermatology*, *19*(8), e1-e8.

Guéniche, A., Dahel, K., Bastien, P., Martin, R., Nicolas, J.F., Breton, L. (2008a). *Vitreoscilla filiformis* bacterial extract to improve the efficacy of emollient used in atopic dermatitis symptoms. *Journal of the European Academy of Dermatology and Venereology*, *22(6)*, 746-747.

Guéniche, A., David, P., Philippe, B., Stephanie, B., Elif, B., Isabelle, C. (2009). Probiotics for photoprotection. *Dermato-Endocrinology*, *1(5)*, 275-279.

Guéniche, A., Hennino, A., Goujon, C., Dahel, K., Bastien, P., Martin, R., Jourdain, R., Breton, L. (2006). Improvement of atopic dermatitis skin symptoms by *Vitreoscilla filiformis* bacterial extract. *European Journal of Dermatology*, *16(4)*, 380-384.

Guéniche, A., Knaudt, B., Schuck, E., Volz, T., Bastien, P., Martin, R., Röcken, M., Breton, L., Biedermann, T. (2008b). Effects of nonpathogenic gram-negative bacterium *Vitreoscilla filiformis* lysate on atopic dermatitis: a prospective, randomized, double-blind, placebo-controlled clinical study. *British Journal of Dermatology*, *159*, 1357-1363.

Gupta, M. A., & Gupta, A. K. (1998). Depression and suicidal ideation in dermatology patients with acne, alopecia areata, atopic dermatitis and psoriasis. *British Journal of Dermatology*, *139*, 846-850.

Gupta, M. A., & Gupta, A. K. (2001). The psychological comorbidity in acne. *Clinics in dermatology*, *19*(3), 360.

Guy, R., Green, M. R., & Kealey, T. (1996a). Modeling acne in vitro. *Journal of investigative dermatology*, *106*(1), 176-182.

Guy, R., Ridden, C., & Kealey, T. (1996b). The improved organ maintenance of the human sebaceous gland: modeling in vitro the effects of epidermal growth factor, androgens, estrogens, 13-cis retinoic acid, and phenol red. *Journal of investigative dermatology*, *106*(3), 454-460.

Hachem, J. P., Crumrine, D., Fluhr, J., Brown, B. E., Feingold, K. R., & Elias, P. M. (2003). pH directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion. *Journal of Investigative Dermatology*, *121*(2), 345-353.

Hachem, J. P., Man, M. Q., Crumrine, D., Uchida, Y., Brown, B. E., Rogiers, V., Roseeuw, D., Feingold, K. R. & Elias, P. M. (2005). Sustained serine proteases activity by prolonged increase in pH leads to degradation of lipid processing enzymes and profound alterations of barrier function and stratum corneum integrity. *Journal of investigative dermatology*, *125*(3), 510-520.

Hacini-Rachinel, F., Gheit, H., Le Luduec, J. B., Dif, F., Nancey, S., & Kaiserlian, D. (2009). Oral probiotic control skin inflammation by acting on both effector and regulatory T cells. *PLoS One*, *4*(3), e4903.

Hajjar, A. M., O'Mahony, D. S., Ozinsky, A., Underhill, D. M., Aderem, A., Klebanoff, S. J., & Wilson, C. B. (2001). Cutting edge: functional interactions between toll-like receptor (TLR) 2 and TLR1 or TLR6 in response to phenol-soluble modulin. *The Journal of Immunology*, *166*(1), 15-19.

Hamer, H. M., Jonkers, D. M. A. E., Venema, K., Vanhoutvin, S. A. L. W., Troost, F. J., & Brummer, R. J. (2008). Review article: the role of butyrate on colonic function. Alimentary pharmacology & therapeutics, 27(2), 104-119.

Hanifin, J. M., Reed, M. L., & Impact Working Group. (2007). A Population-Based Survey of Eczema Prevalence in the United States. Dermatitis, 18(2), 82-91.

Hasper, H. E., Kramer, N. E., Smith, J. L., Hillman, J. D., Zachariah, C., Kuipers, O. P., Kruijff . B & Breukink, E. (2006). An alternative bactericidal mechanism of action for lantibiotic peptides that target lipid II. *Science*, *313*(5793), 1636-1637.

Hauk, P. J., & Leung, D. Y. (2001). Tacrolimus (FK506): new treatment approach in superantigen-associated diseases like atopic dermatitis?. *Journal of allergy and clinical immunology*, *107*(2), 391-392. As cited in: Novak, N., Bieber, T., & Leung, D. Y. (2003). Immune mechanisms leading to atopic dermatitis. *Journal of allergy and clinical immunology*, *112*(6), S128-S139.

Hay, J. B., & Hodgins, M. B. (1974). Metabolism of androgens by human skin in acne. *British Journal of Dermatology*, *91*(2), 123-133.

Hay, J. B., & Hodgins, M. B. (1978). Distribution of androgen metabolizing enzymes in isolated tissues of human forehead and axillary skin. *Journal of Endocrinology*, *79*(1), 29-39.

Hegemann, L., Toso, S. M., Kitay, K., & WEBSTER, C. (1994). Anti-inflammatory actions of benzoyl peroxide: effects on the generation of reactive oxygen species by leucocytes and the activity of protein kinase C and calmodulin. *British Journal of Dermatology*, *130*(5), 569-575.

Hornef, M. W., Wick, M. J., Rhen, M., & Normark, S. (2002). Bacterial strategies for overcoming host innate and adaptive immune responses. *Nature immunology*, *3*(11), 1033-1040.

Imamachi, N., Park, G. H., Lee, H., Anderson, D. J., Simon, M. I., Basbaum, A. I., & Han, S. K. (2009). TRPV1-expressing primary afferents generate behavioral responses to pruritogens via multiple mechanisms. *Proceedings of the National Academy of Sciences*, *106*(27), 11330-11335.

Ingham, E, Walters, C.E, Eady, E.A, et al. (1998). Inflammation in acne vulgaris: failure of skin micro-organisms to modulate keratinocyte interleukin 1 alpha production in vitro. Dermatology, 196, 86–88

Ingham, E., Eady, E. A., Goodwin, C. E., Cove, J. H., & Cunliffe, W. J. (1992). Pro-Inflammatory Levels of Interleukin-1&agr;-Like Bioactivity Are Present in the Majority of Open Comedones in Acne Vulgaris. *Journal of investigative dermatology*, *98*(6), 895-901.

Inoue, K., Koizumi, S., Fuziwara, S., Denda, S., Inoue, K., & Denda, M. (2002). Functional vanilloid receptors in cultured normal human epidermal keratinocytes. *Biochemical and biophysical research communications*, *291*(1), 124-129.

Irvine, A. D., & McLean, W. I. (2006). Breaking the (un) sound barrier: filaggrin is a major gene for atopic dermatitis. *Journal of investigative dermatology*, *126*(6), 1200-1202.

Iwase, T., Uehara, Y., Shinji, H., Tajima, A., Seo, H., Takada, K., Agata, T., Mizunoe, Y. (2010). *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. *Nature*, *465*, 346-349.

James, A.G., Austin, C.J., Cox, D.S., Taylor, D., Calvert, R. (2012). Microbiological and biochemical origins of human axillary odour. *FEMS Microbiology Ecology*, *83(3)*, 527-540.

James, W. D., Berger, T., & Elston, D. (2011). Andrew's diseases of the skin: clinical dermatology. Elsevier Health Sciences.

Janeway Jr, C. A., & Medzhitov, R. (2002). Innate immune recognition. *Annual review of immunology*, *20*(1), 197-216.

Jemec, G. B. E. (2010) ''Standard Grading System for Rosacea.'' *Pathogenesis and Treatment of Acne and Rosacea*. Ed. Zouboulis, C. C., Katsambas, A. D., & Kligman, A. M. *Springer* 648-649

Jeremy, A. H., Holland, D. B., Roberts, S. G., Thomson, K. F., & Cunliffe, W. J. (2003). Inflammatory events are involved in acne lesion initiation. *Journal of Investigative Dermatology*, *121*(1), 20-27.

Kalinin, A. E., Kajava, A. V., & Steinert, P. M. (2002). Epithelial barrier function: assembly and structural features of the cornified cell envelope. *Bioessays*, *24*(9), 789-800.

Kanada, K. N., Nakatsuji, T., & Gallo, R. L. (2012). Doxycycline indirectly inhibits proteolytic activation of tryptic kallikrein-related peptidases and activation of cathelicidin. *Journal of Investigative Dermatology*, *132*(5), 1435-1442.

Karczewski, J., Troost, F. J., Konings, I., Dekker, J., Kleerebezem, M., Brummer, R. J. M., & Wells, J. M. (2010). Regulation of human epithelial tight junction proteins by Lactobacillus plantarum in vivo and protective effects on the epithelial barrier. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *298*(6), G851-G859.

Katsambas, A. D. , Cunliffe, W. J., ,& Zouboulis, C. C., (2010). "Clinical Aspects of Acne Vulgaris'' *Pathogenesis and Treatment of Acne and Rosacea*. Ed. Zouboulis, C. C., Katsambas, A. D., & Kligman, A. M. Springer 223-225

Katsambas, A. D., & Dessinioti, C. (2010). Hormonal therapy for acne: why not as first line therapy? Facts and controversies. *Clinics in dermatology*, *28*(1), 17-23.

Kaufmann, S. H. (2007). The contribution of immunology to the rational design of novel antibacterial vaccines. *Nature Reviews Microbiology*, *5*(7), 491-504.

Kendall, S. N. (2004). Remission of rosacea induced by reduction of gut transit time. *Clinical and experimental dermatology*, *29*(3), 297-299.

Kenyon, F. E. (1966). Psychosomatic aspects of acne: a controlled study. *Transactions of the St. John's Hospital Dermatological Society*, *52*(1), 71.

Kerr, J. R. (1994). Suppression of fungal growth exhibited by Pseudomonas aeruginosa. *Journal of clinical microbiology*, *32*(2), 525-527.

Kerschenlohr, K., Decard, S., Przybilla, B., & Wollenberg, A. (2003). Atopy patch test reactions show a rapid influx of inflammatory dendritic epidermal cells in patients with extrinsic atopic dermatitis and patients with intrinsic atopic dermatitis. *Journal of allergy and clinical immunology*, *111*(4), 869-874.

Kezic, S., O'Regan, G. M., Yau, N., Sandilands, A., Chen, H., Campbell, L. E., ... & Irvine, A. D. (2011). Levels of filaggrin degradation products are influenced by both filaggrin genotype and atopic dermatitis severity. *Allergy*, *66*(7), 934-940.

Kim, H.M., Lee, D.E., Park, S.D., Kim, Y., Kim, Y.J., Jeong, J.W., Jang, S.S., Ahn, Y., Sim, J., Huh, C., Chung, D.K., Lee, J. (2014). Oral administration of *Lactobacillus plantarum* HY7714 protects hairless mouse against ultraviolet B-induced photoaging. *Journal of Microbiology and Biotechnology*, *24(11)*, 1583-1591.

Kim, J. Y., Kwon, J. H., Ahn, S. H., Lee, S. I., Han, Y. S., Choi, Y. O., Lee, S. Y., Ahn, K. M. & Ji, G. E. (2010). Effect of probiotic mix (Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus acidophilus) in the primary prevention of eczema: a double-blind, randomized, placebo-controlled trial. *Pediatric Allergy and Immunology*, *21*(2p2), e386-e393.

Kisich, K. O., Carspecken, C. W., Fiéve, S., Boguniewicz, M., & Leung, D. Y. (2008). Defective killing of *Staphylococcus aureus* in atopic dermatitis is associated with reduced mobilization of human β-defensin-3. *Journal of Allergy and Clinical Immunology*, *122*(1), 62-68.

Kisich, K. O., Howell, M. D., Boguniewicz, M., Heizer, H. R., Watson, N. U., & Leung, D. Y. (2007). The constitutive capacity of human keratinocytes to kill Staphylococcus aureus is dependent on β-defensin 3. *Journal of Investigative Dermatology*, *127*(10), 2368-2380.

Klein, G., Pack, A., Bonaparte, C. & Reuter, G. (1998). Taxonomy and Physiology of Probiotic Lactid Acid Bacteria. *Internat. J. Food Microbiol. 41,* 103-125.

Kohmoto, T. (1988). Effect of isomalto-oligosaccharides on human fecal flora. *Bifidobact. Microflora*, *7*, 61-69

Kong, H.H., Segre, J.A. (2012). Skin microbiome: looking back to move forward. *Journal of Investigative Dermatology*, *132*, 933-939.

Koo, J. (1995). The psychosocial impact of acne: patients' perceptions. *Journal of the American Academy of Dermatology*, *32*(5), S26-S30.

Korczak, D. (1989). Psychische Situation der Akne-Patienten. Persönlichkeitsstruktur und Arzt-Patient-Beziehung. *Fortschr Med*, *107*, 309-313.

Krutmann, J. (2009). Pre- and probiotics for human skin. *Journal of Dermatological Science*, *54*, 1-5.

Kurokawa, I., Danby, F. W., Ju, Q., Wang, X., Xiang, L. F., Xia, L., Chen, W., Nagy, I., Picardo, M., Suh, D. H., Ganceiciene, R. Scagen, S., Tastsou, T., & Zouboulis, C. C. (2009). New developments in our understanding of acne pathogenesis and treatment. *Experimental dermatology*, *18*(10), 821-832.

La Colla, L., Mangano, A., Mangano, A., Albertin, A. (2009). Effects of nonpathogenic gram-negative bacterium *Vitreoscilla filiformis* lysate on atopic dermatitis: a prospective, randomized, double-blind, placebo-controlled clinical study. Does this make a real difference? *British Journal of Dermatology*, *161(2)*, 477-478.

Lacey, N., & Powell, F. C., (2010). ''Rosacea and *Demodex folliculorum*'' *Pathogenesis and Treatment of Acne and Rosacea*. Ed. Zouboulis, C. C., Katsambas, A. D., & Kligman, A. M. *Springer* 628-637

Lacey, N., Delaney, S., Kavanagh, K., & Powell, F. C. (2007). Mite-related bacterial antigens stimulate inflammatory cells in rosacea. *British Journal of Dermatology*, *157*(3), 474-481.

Lai, Y., Cogen, A.L., Radek, K.A., Park, H.J., MacLeod, D.T., Leichtle, A., Ryan, A.F., Di Nardo, A., Gallo, R.L. (2010). Activation of TLR2 by a small molecule produced by *Staphylococcus epidermidis* increases antimicrobial defense against bacterial skin infections. *Journal of Investigative Dermatology*, *130*, 2211-2221.

Laughton, J. M., Devillard, E., Heinrichs, D. E., Reid, G., & McCormick, J. K. (2006). Inhibition of expression of a staphylococcal superantigen-like protein by a soluble factor from Lactobacillus reuteri. *Microbiology*, *152*(4), 1155-1167.

Layton, A. M., (2010) "The Leeds Acne Grading Technique'' *Pathogenesis and Treatment of Acne and Rosacea*. Ed. Zouboulis, C. C., Katsambas, A. D., & Kligman, A. M. *Springer* 317-324

Lazaridou, E., Giannopoulou, C., Fotiadou, C., Vakirlis, E., Trigoni, A., & Ioannides, D. (2011). The potential role of microorganisms in the development of rosacea. *JDDG: Journal der Deutschen Dermatologischen Gesellschaft*, *9*(1), 21-25.

Lee, P. P., Ferguson Jr, D. A., & Sarubbi, F. A. (2005). Corynebacterium striatum: an underappreciated community and nosocomial pathogen. *Journal of Infection*, *50*(4), 338-343.

Lee, S. E., Kim, J. M., Jeong, S. K., Jeon, J. E., Yoon, H. J., Jeong, M. K., & Lee, S. H. (2010). Protease-activated receptor-2 mediates the expression of inflammatory cytokines, antimicrobial peptides, and matrix metalloproteinases in keratinocytes in response to Propionibacterium acnes. *Archives of dermatological research*, *302*(10), 745-756.

Lee, W. J., Jung, H. D., Lee, H. J., Kim, B. S., & Lee, S. J. (2008). Influence of substance-P on cultured sebocytes. *Archives of dermatological research*, *300*(6), 311-316.

Lee, Y. A., Wahn, U., Kehrt, R., Tarani, L., Businco, L., Gustafsson, D., ... & Reis, A. (2000). A major susceptibility locus for atopic dermatitis maps to chromosome 3q21. *Nature genetics*, *26*(4), 470-473.

Leeming, J. P., Holland, K. T., & Cuncliffe, W. J. (1988). The microbial colonization of inflamed acne vulgaris lesions. *British journal of dermatology*, *118*(2), 203-208.

Letawe, C., Boone, M., & Piérard, G. E. (1998). Digital image analysis of the effect of topically applied linoleic acid on acne microcomedones. *Clinical and experimental dermatology*, *23*(2), 56-58.

Leung, D. Y. (2003). Infection in atopic dermatitis. *Current opinion in pediatrics*, *15*(4), 399-404.

Leung, D. Y. M. "Atopic dermatitis: new insights and opportunities for therapeutic intervention." *Journal of Allergy and Clinical Immunology* 105.5 (2000): 860-876.

Leung, D. Y., Harbeck, R., Bina, P., Reiser, R. F., Yang, E., Norris, D. A., ... & Sampson, H. A. (1993). Presence of IgE antibodies to staphylococcal exotoxins on the skin of patients with atopic dermatitis. Evidence for a new group of allergens. *Journal of Clinical Investigation*, *92*(3), 1374.

Lew, L.-C., Liong, M.-T. (2013) Bioactives from probiotics for dermal health: functions and benefits. *Journal of Applied Microbiology*, *114*, 1241-1253.

Lew, L.-C., Liong, M.-T., Gan, C.-Y. (2012). Growth optimization of *Lactobacillus rhamnosus* FTDC 8313 and the production of putative dermal bioactives in the presence of manganese and magnesium ions. *Journal of Applied Microbiology*, *114*, 526-535.

Leyden, J. J., Del Rosso, J. Q., & Webster, G. F. (2009). Clinical considerations in the treatment of acne vulgaris and other inflammatory skin disorders: a status report. *Dermatologic clinics*, *27*(1), 1-15.

Leyden, J. J., Marples, R. R., & Kligman, A. M. (1974). Staphylococcus aureus in the lesions of atopic dermatitis. *British Journal of Dermatology*, *90*(5), 525-525.

Leyden, J. J., McGinley, K. J., Mills, O. H., & Kligman, A. M. (1975). Propionibacterium levels in patients with and without acne vulgaris. *Journal of Investigative Dermatology*, *65*(4), 382-384.

Lim, K. M., & Park, Y. H. (2012). Development of PAC-14028, a novel transient receptor potential vanilloid type 1 (TRPV1) channel antagonist as a new drug for refractory skin diseases. *Archives of pharmacal research*, *35*(3), 393-396.

Lin, C. S., Chang, C. J., Lu, C. C., Martel, J., Ojcius, D. M., Ko, Y. F., Young, J. D. & Lai, H. C. (2014). Impact of the Gut Microbiota, Prebiotics, and Probiotics on Human Health and Disease. *Biomed. J. 37*(5), 259-268.

Lina, G., Boutite, F., Tristan, A., Bes, M., Etienne, J., & Vandenesch, F. (2003). Bacterial competition for human nasal cavity colonization: role of Staphylococcal *agr* alleles. *Applied and Environmental Microbiology*, *69(1)*, 18-23.

Liu, C. H., Lee, S. M., VanLare, J. M., Kasper, D. L., & Mazmanian, S. K. (2008). Regulation of surface architecture by symbiotic bacteria mediates host colonization. *Proceedings of the National Academy of Sciences*, *105*(10), 3951-3956.

Lovejoy, E. D., & Hastings, T. W. (1911). Isolation and growth of the acne bacillus. *J. cutan. Dis*, *29*(80), 191.

Lowe, N. J., Behr, K. L., Fitzpatrick, R., Goldman, M., & Ruiz-Esparza, J. (1991). Flash lamp pumped dye laser for rosacea-associated telangiectasia and erythema. *The Journal of dermatologic surgery and oncology*, *17*(6), 522-525.

Lucky, M., A. (1998). A Review of Infantile and PediatricAcne. *Dermatology*, *196*(1), 95-97.

Mahe, Y.F., Martin, R., Aubert, L., Billoni, N., Collin, C., Pruche, F., Bastien, P., Drost, S.S., Lane, A.T., Meybeck, A. (2006). Induction of the skin endogenous protective mitochondrial MnSOD by *Vitreoscilla filiformis* extract. *International Journal of Cosmetic Science*, *28(4)*, 277-287.

Mahe, Y.F., Perez, M.-J., Tacheau, C., Fanchon, C., Martin, R., Rousset, F., Seite, S. (2013). A new *Vitreoscilla filiformis* extract grown on spa water-enriched medium activates endogenous cutaneous antioxidant and antimicrobial defenses through a potential Toll-like receptor 2/protein kinase C, zeta transduction pathway. *Clinical, Cosmetic and investigational Dermatology*, *6*, 191-196.

Maisonneuve, H., Cambazard, F., Levy, E., & Thivolet, J. (1987). Évaluation du nombre et du côut des acnes sévères en France. In *Annales de dermatologie et de vénéréologie* (Vol. 114, No. 10, pp. 1203-1209). Masson.

Male, V., Nisoli, I., Gascoyne, D. M., & Brady, H. J. (2012). E4BP4: an unexpected player in the immune response. *Trends in immunology*, *33*(2), 98-102.

Mallon, E., Newton, J. N., Klassen, A., Stewart-Brown, S. L., Ryan, T. J., & Finlay, A. Y. (1999). The quality of life in acne: a comparison with general medical conditions using generic questionnaires. *British Journal of Dermatology*, *140*, 672-676.

Maniati, E., Soper, R., & Hagemann, T. (2010). Up for Mischief? IL-17/Th17 in the tumour microenvironment. *Oncogene*, *29*(42), 5653-5662.

Marteau, P., & Boutron-Ruault, M. C. (2002). Nutritional advantages of probiotics and prebiotics. *British Journal of Nutrition*, *87*(S2), S153-S157.

Matsuda, H., Watanabe, N., Geba, G. P., Sperl, J., Tsudzuki, M., Hiroi, J., Ushio, H., Askenase, P. W., & Ra, C. (1997). Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. *International Immunology*, *9*(3), 461-466.

Matsuguchi, T., Takagi, A., Matsuzaki, T., Nagaoka, M., Ishikawa, K., Yokokura, T., & Yoshikai, Y. (2003). Lipoteichoic acids from *Lactobacillus* strains elicit strong tumor necrosis factor alpha-inducing activities in macrophages through Toll-like receptor 2. *Clinical and diagnostic laboratory immunology*, *10*(2), 259-266.

Mayer-da-Silva, A., Gollnick, H., Detmar, M., Gassmüller, J., Parry, A., Müller, R., & Orfanos, C. E. (1988). Effects of azelaic acid on sebaceous gland, sebum excretion rate and keratinization pattern in human skin. An in vivo and in vitro study. *Acta dermato-venereologica. Supplementum*, *143*, 20-30.

McAleer, M. A., Powell, F. C., (2010) ''Rosacea and Neuropeptides'' *Pathogenesis and Treatment of Acne and Rosacea*. Ed. Zouboulis, C. C., Katsambas, A. D., & Kligman, A. M. *Springer* 622

McCall, C. A., & Cohen, J. J. (1991). Programmed cell death in terminally differentiating keratinocytes: role of endogenous endonuclease. *Journal of investigative dermatology*, *97*(1), 111-114.

Meckfessel, M.H., Brandt, S. (2014). The structure, function, and importance of ceramides in skin and their use as therapeutic agents in skin-care products. *Journal of the American Academy of Dermatology*, *71(1)*, 177-184.

Medzhitov, R. (2001). Toll-like receptors and innate immunity. *Nature Reviews Immunology*, *1*(2), 135-145.

Mikelsaar, M., & Zilmer, M. (2009). Lactobacillus fermentum ME-3-an antimicrobial and antioxidative probiotic. *Microbial ecology in health and disease*, *21*(1), 1-27.

Mills, O. H., Kligman, A. M., Pochi, P., & Comite, H. (1986). Comparing 2.5%, 5%, and 10% benzoyl peroxide on inflammatory acne vulgaris. *International journal of dermatology*, *25*(10), 664-667.

Mitsuoka, T., Hidaka, H., & Eida, T. (1987). Effect of fructo-oligosaccharides on intestinal microflora. *Food/Nahrung*, *31*(5-6), 427-436.

Morar, N., Willis-Owen, S. A., Moffatt, M. F., & Cookson, W. O. (2006). The genetics of atopic dermatitis. Journal of Allergy and Clinical Immunology, 118(1), 24-34.

Nagy, I., Pivarcsi, A., Kis, K., Koreck, A., Bodai, L., McDowell, A., Selmann, H., Patrick, S., Zouboulis, C.C., Kemény, L. (2006). Propionibacterium acnes and lipopolysaccharide induce the expression of antimicrobial peptides and proinflammatory cytokines/chemokines in human sebocytes. *Microbes and infection*, *8*(8), 2195-2205.

Narayanan, S., Hùnerbein, A., Getie, M., Jäckel, A., & Neubert, R. H. (2007). Scavenging properties of metronidazole on free oxygen radicals in a skin lipid model system. *Journal of pharmacy and pharmacology*, *59*(8), 1125-1130.

Nathan, A. T., Peterson, E. A., Chakir, J., & Wills-Karp, M. (2009). Innate immune responses of airway epithelium to house dust mite are mediated through β-glucan–dependent pathways. *Journal of Allergy and Clinical Immunology*, *123*(3), 612-618.

Nathan, C. (2006). Neutrophils and immunity: challenges and opportunities. *Nature Reviews Immunology*, *6*(3), 173-182.

Ní Raghallaigh, S., & Powell, F. C. (2014). Epidermal hydration levels in patients with rosacea improve after minocycline therapy. *British Journal of Dermatology*.

Nicholson, K., Abramova, L., Chren, M. M., Yeung, J., Chon, S. Y., & Chen, S. C. (2007). A pilot quality-of-life instrument for acne rosacea. *Journal of the American Academy of Dermatology*, *57*(2), 213-221.

Niemeier, V., Kupfer, J., Demmelbauer-Ebner, M., Stangier, U., Effendy, I., & Gieler, U. (1998). Coping with acne vulgaris. *Dermatology*, *196*(1), 108-115.

Nijsten, T., Rombouts, S., & Lambert, J. (2007). Acne is prevalent but use of its treatments is infrequent among adolescents from the general population. *Journal of the European Academy of Dermatology and Venereology*, *21*(2), 163-168.

Nikkari, T. (1974). Comparative chemistry of sebum. *Journal of Investigative Dermatology*, *62*(3), 257-267.

Nizet, V., Ohtake, T., Lauth, X., Trowbridge, J., Rudisill, J., Dorschner, R. A., Pestonjamasp, V., Piraino, K., Huttne, K., & Gallo, R. L. (2001). Innate antimicrobial peptide protects the skin from invasive bacterial infection. *Nature*, *414*(6862), 454-457.

Norris, J. F. B., & Cunliffe, W. J. (1988). A histological and immunocytochemical study of early acne lesions. *British Journal of Dermatology*, *118*(5), 651-659.

Ochsendorf, R., (2010). "Oral Antibiotics" *Pathogenesis and Treatment of Acne and Rosacea*. Ed. Zouboulis, C. C., Katsambas, A. D., & Kligman, A. M. Springer 450-457

Ong, P. Y., Ohtake, T., Brandt, C., Strickland, I., Boguniewicz, M., Ganz, T., ... & Leung, D. Y. (2002). Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *New England Journal of Medicine*, *347*(15), 1151-1160.

Oranje, A.P., Glazenburg, E.J., Wolkerstorfer, A. and De Waard-van der Spek, F.B. (2007), Practical issues on interpretation of scoring atopic dermatitis: the SCORAD index, objective SCORAD and the three-item severity score. British Journal of Dermatology, 157: 645–648. doi: 10.1111/j.1365-2133.2007.08112.x

Ottaviani, M., Alestas, T., Flori, E., Mastrofrancesco, A., Zouboulis, C. C., & Picardo, M. (2006). Peroxidated squalene induces the production of inflammatory mediators in HaCaT keratinocytes: a possible role in acne vulgaris. *Journal of investigative dermatology*, *126*(11), 2430-2437.

Ottaviani, M., Camera, E., & Picardo, M. (2010). Lipid mediators in acne. *Mediators of inflammation*, *2010*.

Otto, M. (2009). *Staphylococcus epidermidis* - the 'accidental' pathogen. *Nature Reviews Microbiology*, *7*, 555-567.

Öztas, M. O., Balk, M., Ögüs, E., Bozkurt, M., Ögüs, I. H., & Özer, N. (2003). The role of free oxygen radicals in the aetiopathogenesis of rosacea. *Clinical and experimental dermatology*, *28*(2), 188-192.

Palmer, C. N., Irvine, A. D., Terron-Kwiatkowski, A., Zhao, Y., Liao, H., Lee, S. P., ... & McLean, W. I. (2006). Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nature genetics*, *38*(4), 441-446.

Pamer, E. G. (2007). Immune responses to commensal and environmental microbes. *Nature immunology*, *8*(11), 1173-1178.

Park, B., Iwase, T., Liu, G.Y. (2011). Intranasal application of *S. epidermidis* prevents colonization by Methicillin-resistant *Staphylococcus aureus* in mice. *PLoS ONE*, *6(10)*, e25880.

Parnham, M. J. (2011). Immunomodulatory approaches to the treatment of infections. *Infektološki Glasnik*, *31*(1), 15-27.

Parodi, A., Paolino, S., Greco, A., Drago, F., Mansi, C., Rebora, A., Parodi, A., & Savarino, V. (2008). Small intestinal bacterial overgrowth in rosacea: clinical effectiveness of its eradication. *Clinical Gastroenterology and Hepatology*, *6*(7), 759-764. As cited in Lazaridou, E., Giannopoulou, C., Fotiadou, C., Vakirlis, E., Trigoni, A., & Ioannides, D. (2011). The potential role of microorganisms in the development of rosacea. *JDDG: Journal der Deutschen Dermatologischen Gesellschaft*, *9*(1), 21-25.

Pelle, M. T., Crawford, G. H., & James, W. D. (2004). Rosacea: II. therapy. *Journal of the American Academy of Dermatology*, *51*(4), 499-512.

Peus, D., Vasa, R. A., Beyerle, A., Meves, A., Krautmacher, C., & Pittelkow, M. R. (1999). UVB activates ERK1/2 and p38 signaling pathways via reactive oxygen species in cultured keratinocytes. *Journal of investigative dermatology*, *112*(5), 751-756.

Picardi, A., Abeni, D., Melchi, C. F., Puddu, P., & Pasquini, P. (2000). Psychiatric morbidity in dermatological outpatients: an issue to be recognized. *British Journal of dermatology*, *143*(5), 983-991.

Picardo, M., Ottaviani, M., Camera, E., & Mastrofrancesco, A. (2009). Sebaceous gland lipids. *Dermatoendocrinol*, *1*(2), 68-71.

Pimentel, M., Chow, E. J., Hallegua, D., Wallace, D., & Lin, H. C. (2001). Small intestinal bacterial overgrowth: a possible association with fibromyalgia. *Journal of Musculoskelatal Pain*, *9*(3), 105-113.

Pivarcsi, A., Bodai, L., Réthi, B., Kenderessy-Szabó, A., Koreck, A., Széll, M., Beer, Z., Bata-Csorgoo, Z., Magocsi, M., Rajnavolgyi, E., Dobozy, A., & Kemény, L. (2003). Expression and function of Toll-like receptors 2 and 4 in human keratinocytes. *International immunology*, *15*(6), 721-730.

Plewig G., & Kligman A. M. (2000). *Acne and rosacea*. *3rd ed. Berlin: Springer,* 286-287

Pochi, P. E., & Strauss, J. S. (1964). Sebum Production, Casual Sebum Levels, Titratable Acidity of Sebum, and Urinary Fractional 17-Ketosteroid Excretion in Males with Acne1. *Journal of Investigative Dermatology*, *43*(5), 383-388.

Pochi, P. E., & Strauss, J. S. (1974). ENDOCRINOLOGIC CONTROL TO THE DEVELOPMENT AND ACTIVITY OF THE HUMAN SEBACEOUS GLAND. *Journal of Investigative Dermatology*, *62*(3), 191-201.

Pochi, P. E., Downing, D. T., & Strauss, J. S. (1970). Sebaceous gland response in man to prolonged total caloric deprivation. *Journal of Investigative Dermatology*, *55*(5), 303-309.

Pochi, P. E., Strass, J. S., Rao, G. S., Sada, I. R., Forchiell, E., & Dorfarm, R. I. (1965). Plasma testosterone and estrogen levels, urine testosterone excretion, and sebum production in males with acne vulgaris. *The Journal of Clinical Endocrinology & Metabolism*, *25*(12), 1660-1664.

Poelstra, K., Bakker, W. W., Klok, P. A., Kamps, J. A., Hardonk, M. J., & Meijer, D. K. (1997). Dephosphorylation of endotoxin by alkaline phosphatase in vivo. *The American journal of pathology*, *151*(4), 1163.

Poli, F., Dreno, B., & Verschoore, M. (2001). An epidemiological study of acne in female adults: results of a survey conducted in France. *Journal of the European Academy of Dermatology and Venereology*, *15*(6), 541-545.

Powell, F. C., Corbally, N., & Powell, D. (1993). Substance P and rosacea. *Journal of the American Academy of Dermatology*, *28*(1), 132-133.

Prince, T., McBain, A.J., & O'Neill, C.A. (2012) *Lactobacillus reuteri* protects epidermal keratinocytes from *Staphylococcus aureus*-induced cell death by competitive exclusion. *Applied and Environmental Microbiology*, *78(15)*, 5119-5126.

Purdy, S. (2006). Acne vulgaris. *Clinical evidence*, (15), 2183-2201.

Raghallaigh, S. N., & Powell, F. C. (2009) The cutaneous microenvironment in papulopustular rosacea. In *BRITISH JOURNAL OF DERMATOLOGY* , 161, 25-25. Cited in Lacey, N., & Powell, F. C. (2010) ''Rosacea and *Demodex folliculorum*'' *Pathogenesis and Treatment of Acne and Rosacea*. Ed. Zouboulis, C. C., Katsambas, A. D., & Kligman, A. M. *Springer* 628-637

Ramanathan, S., & Hebert, A. A. (2011). Management of acne vulgaris. *Journal of Pediatric Health Care*, *25*(5), 332-337.

RASMUSSEN, J. E. (1977). Diet and acne. *International journal of dermatology*, *16*(6), 488-492.

Rawlings, A. V., Scott, I. R., Harding, C. R., & Bowser, P. A. (1994). Stratum corneum moisturization at the molecular level. *Journal of investigative dermatology*, *103*(5), 731-740.

Reichert, U., Michel, S., & Schmidt, R. (1993). The cornified envelope: a key structure of terminally differentiating keratinocytes. Molecular Biology of the Skin: the keratinocyte, 107-150.

Reid, G., Younes, J. A., Van der Mei, H. C., Gloor, G. B., Knight, R., & Busscher, H. J. (2011). Microbiota restoration: natural and supplemented recovery of human microbial communities. *Nature Reviews Microbiology*, *9*(1), 27-38.

Rosner, H. Grimmecke, H., Knirel, Y.A., Shashkov, S. (1992) Hyaluronic acid and a (1→4)-*β*-D-xylan, extracellular saccharides of *Pasteurella multicoda* (Carter type A) strain 880. *Carbohydrate Research*, *223*, 329-333.

Rundhaug, J. E. (2005). Matrix metalloproteinases and angiogenesis. *Journal of cellular and molecular medicine*, *9*(2), 267-285.

Ryu, J. H., Kim, S. H., Lee, H. Y., Bai, J. Y., Nam, Y. D., Bae, J. W., Dong. G. L, Seung. C. S, Eun-Mi. H & Lee, W. J. (2008). Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in Drosophila. *science*, *319*(5864), 777-782.

Sandilands, A., Sutherland, C., Irvine, A. D., & McLean, W. I. (2009). Filaggrin in the frontline: role in skin barrier function and disease. *Journal of cell science*, *122*(9), 1285-1294.

Sanford, J.A., Gallo, R.L. (2013). Functions of the skin microbiota in health and disease. *Seminars in Immunology*, *25*, 370-377.

Schachner, L., Field, T., Hernandez-Reif, M., Duarte, A. M., & Krasnegor, J. (1998). Atopic dermatitis symptoms decreased in children following massage therapy. *Pediatric dermatology*, *15*(5), 390-395.

Scharschmidt, T.C., Fischbach, M.A. (2013). What lives on our skin: ecology, genomics and therapeutic opportunities of the skin microbiome. *Drug Discovery Today: Disease Mechanisms*, *10(3-4)*, e83-e89.

Schauber, J., Dorschner, R. A., Yamasaki, K., Brouha, B., & Gallo, R. L. (2006). Control of the innate epithelial antimicrobial response is cell-type specific and dependent on relevant microenvironmental stimuli. *Immunology*, *118*(4), 509-519.

Schmid-Ott, G., Jaeger, B., Adamek, C., Koch, H., Lamprecht, F., Kapp, A., & Werfel, T. (2001a). Levels of circulating CD8+ T lymphocytes, natural killer cells, and eosinophils increase upon acute psychosocial stress in patients with atopic dermatitis. Journal of allergy and clinical immunology, 107(1), 171-177.

Schmid-Ott, G., Jaeger, B., Kapp, A., & Werfel, T. (2001b). Different expression of cytokine and membrane molecules by circulating lymphocytes on acute mental stress in patients with atopic dermatitis in comparison with healthy controls. Journal of allergy and clinical immunology, 108(3), 455-462.

Schommer, N.N., Gallo, R.L. (2013). Structure and function of the human skin microbiome. *Trend in Microbiology*, *21(12)*, 660-668.

Schultz, L. F., Holm, N. V., & Henningsen, K. (1986). Atopic dermatitis: a genetic-epidemiologic study in a population-based twin sample. *Journal of the American Academy of Dermatology*, *15*(3), 487-494. as cited in: Strachan, D. P., Wong, H. J., & Spector, T. D. (2001). Concordance and interrelationship of atopic diseases and markers of allergic sensitization among adult female twins. Journal of allergy and clinical immunology, 108(6), 901-907.

Seneschal, J., Clark, R. A., Gehad, A., Baecher-Allan, C. M., & Kupper, T. S. (2012). Human epidermal Langerhans cells maintain immune homeostasis in skin by activating skin resident regulatory T cells. *Immunity*, *36*(5), 873-884.

Shaheen, B., & Gonzalez, M. (2011). A microbial aetiology of acne: what is the evidence?. *British Journal of Dermatology*, *165*(3), 474-485.

Silverberg, J. I., Hanifin, J., & Simpson, E. L. (2013). Climatic factors are associated with childhood eczema prevalence in the United States. Journal of Investigative Dermatology, 133(7), 1752-1759.

Simonart, T., & Dramaix, M. (2005). Treatment of acne with topical antibiotics: lessons from clinical studies. *British journal of dermatology*, *153*(2), 395-403.

Singh, P. A. R. A. M. J. I. T., Sihorkar, V., Jaitely, V. I. K. A. S., Kanaujia, P., & Vyas, S. P. (2000). Pilosebaceous unit: anatomical considerations and drug delivery opportunities. *Indian Journal of Pharmacology*, *32*(5), 269-281.

Skidmore, R., Kovach, R., Walker, C., Thomas, J., Bradshaw, M., Leyden, J., James, M. D., Powala, B.S & Ashley, R. (2003). Effects of subantimicrobial-dose doxycycline in the treatment of moderate acne. *Archives of dermatology*, *139*(4), 459-464.

Sloan, B., & Scheinfeld, N. (2008). The use and safety of doxycycline hyclate and other second-generation tetracyclines.

Slominski, A., & Wortsman, J. (2000). Neuroendocrinology of the Skin 1. *Endocrine reviews*, *21*(5), 457-487.

Smith, R. N., Mann, N. J., Braue, A., Mäkeläinen, H., & Varigos, G. A. (2007). A low-glycemic-load diet improves symptoms in acne vulgaris patients: a randomized controlled trial. *The American journal of clinical nutrition*, *86*(1), 107-115.

Smithard, A., Glazebrook, C., & Williams, H. C. (2001). Acne prevalence, knowledge about acne and psychological morbidity in mid-adolescence: a community-based study. *British Journal of Dermatology*, *145*(2), 274-279.

Soccol, C. R., Vandenberghe, L. P. S., Spier, M. R., Medeiros, A. B. P., Yamaguishi, C. T., Lindner, J. D. D., Pandey, A. & Soccol, V. T. (2010). The Potential of Probiotics: A Review. *Food Technol. Biotechnol. 48*(4), 413-434.

Spergel, J. M., Mizoguchi, E., Brewer, J. P., Martin, T. R., Bhan, A. K., & Geha, R. S. (1998). Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. *Journal of Clinical Investigation*, *101*(8), 1614.

Steinert, P. M., & Marekov, L. N. (1995). The proteins elafin, filaggrin, keratin intermediate filaments, loricrin, and small proline-rich proteins 1 and 2 are isodipeptide cross-linked components of the human epidermal cornified cell envelope. *Journal of Biological Chemistry*, *270*(30), 17702-17711.

Stenn, K. S., & Paus, R. (2001). Controls of hair follicle cycling. *Physiological reviews*, *81*(1), 449-494.

Stewart, M. E., Downing, D. T., Cook, J. S., Hansen, J. R., & Strauss, J. S. (1992). Sebaceous gland activity and serum dehydroepiandrosterone sulfate levels in boys and girls. *Archives of dermatology*, *128*(10), 1345-1348.

Stiles, M. E. & Holzapfel, W. H. (1997). Lactic Acid Bacteria of Foods and their Current Taxonomy. *Internat. J. Food Microbiol. 36*, 1-29.

Strachan, D. P., Wong, H. J., & Spector, T. D. (2001). Concordance and interrelationship of atopic diseases and markers of allergic sensitization among adult female twins. Journal of allergy and clinical immunology, 108(6), 901-907.

Strauss, J. S., & Kligman, A. M. (1960). The pathologic dynamics of acne vulgaris. *Archives of Dermatology*, *82*(5), 779-790.

Strauss, J. S., Krowchuk, D. P., Leyden, J. J., Lucky, A. W., Shalita, A. R., Siegfried, E. C., Thiboutot, D. M., Voorhees, A. S., Beutner, K. A., Sieck, C. K., & Bhushan, R. (2007). Guidelines of care for acne vulgaris management. *Journal of the American Academy of Dermatology*, *56*(4), 651-663.

Sugimoto, S., Iwamoto, T., Takada, K., Okuda, K., Tajima, A., Iwase, T., Mizunoe, Y. (2013) *Journal of Bacteriology*, *195(8)*, 1645-1655.

Suh, Y. G., & Oh, U. (2005). Activation and activators of TRPV1 and their pharmaceutical implication. *Current pharmaceutical design*, *11*(21), 2687-2698.

Sutherland, R., Boon, R. J., Griffin, K. E., Masters, P. J., Slocombe, B., & White, A. R. (1985). Antibacterial activity of mupirocin (pseudomonic acid), a new antibiotic for topical use. *Antimicrobial agents and chemotherapy*, *27*(4), 495-498.

Sutherland, R., Boon, R. J., Griffin, K. E., Masters, P. J., Slocombe, B., & White, A. R. (1985). Antibacterial activity of mupirocin (pseudomonic acid), a new antibiotic for topical use. *Antimicrobial agents and chemotherapy*, *27*(4), 495-498.

Tabata, N., Tagami, H., & Kligman, A. M. (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 DERMATOVENEREOLOGICA-STOCKHOLM-*, *78*, 244-247.

Takeda, K., & Akira, S. (2005). Toll-like receptors in innate immunity. *International immunology*, *17*(1), 1-14.

Tan, H. H. (2004). Topical antibacterial treatments for acne vulgaris. *American journal of clinical dermatology*, *5*(2), 79-84.

Tan, J., & Berg, M. (2013). Rosacea: Current state of epidemiology. *Journal of the American Academy of Dermatology*, *69*(6), S27-S35.

Tanghetti, E. A. (2013). The role of inflammation in the pathology of acne. *The Journal of clinical and aesthetic dermatology*, *6*(9), 27.

Tanno, O., Ota, Y., Kitamura, N., Katsube, T., & Inoue, S. (2000). Nicotinamide increases biosynthesis of ceramides as well as other stratum corneum lipids to improve the epidermal permeability barrier. *British Journal of Dermatology*, *143*(3), 524-531.

Taylor, B., Wadsworth, M., Wadsworth, J., & Peckham, C. (1984). Changes in the reported prevalence of childhood eczema since the 1939-45 war. The Lancet, 324(8414), 1255-1257.

Thiboutot, D, Knaggs, H, Galiland, K, et al. (1997). Activity of type 1 5 alpha reductase is greater in the follicular infrainfundibulum compared with the epidermis. Br J Dermatol, 136, 166–71.

Thiboutot, D. (2002). Acne: 1991-2001. Thiboutot DM, Knaggs H, Galiland K, et al. Activityof type 1 5 alpha reductase is greater in the follicular infrainfundibulum compared with the epidermis. Br J Dermatol. 1997;136:166–71., *47*(1), 109-117.

Thiboutot, D., Gollnick, H., Bettoli, V., Dréno, B., Kang, S., Leyden, J. J., Shalita, A. R., Berson. D., & Wolf Jr, J. (2009). New insights into the management of acne: an update from the Global Alliance to Improve Outcomes in Acne group. *Journal of the American Academy of Dermatology*, *60*(5), S1-S50.

Thielitz, A., & Gollnick, H. (2008). Topical retinoids in acne vulgaris. *American journal of clinical dermatology*, *9*(6), 369-381.

Thielitz, A., Reinhold, D., Vetter, R., Bank, U., Helmuth, M., Hartig, R. ,Neubert, K. Faust, J. Zouboulis, C. Z., Ansorge, S. & Gollnick, H. (2006). Inhibitors of dipeptidyl peptidase IV and aminopeptidase N target major pathogenetic steps in acne initiation. *Journal of Investigative Dermatology*, *127*(5), 1042-1051.

Tojo R., Suárez, A., Clemente, M. G., Reyes-Gavilán, C. G., Margolles, A., Gueimonde, M., Ruas-Madiedo, P. (2014). Intestinal Microbiota in Health and Disease: Role of Bifidobacteria in gut homeostasis. *World J. Gastroenterol. 20*(41), 15163-15176.

Toyoda, M., Nakamura, M., & Moroashi, M. (2002a). Neuropeptides and sebaceous glands. *European Journal of Dermatology*, *12*(5), 422-7.

Toyoda, M., Nakamura, M., Makino, T., Kagoura, M., & Morohashi, M. (2002b). Sebaceous glands in acne patients express high levels of neutral endopeptidase. *Experimental dermatology*, *11*(3), 241-247.

Trautmann, A., Akdis, M., Kleemann, D., Altznauer, F., Simon, H. U., Graeve, T., ... & Akdis, C. A. (2000). T cell–mediated Fas-induced keratinocyte apoptosis plays a key pathogenetic role in eczematous dermatitis. *The Journal of clinical investigation*, *106*(1), 25-35.

Trivedi, N. R., Gilliland, K. L., Zhao, W., Liu, W., & Thiboutot, D. M. (2006). Gene array expression profiling in acne lesions reveals marked upregulation of genes involved in inflammation and matrix remodeling. *Journal of investigative dermatology*, *126*(5), 1071-1079.

Tsilingiri, K. & Rescigno, M. (2013). Postbiotics: what else? *Benef. Microb. 4*(1), 101-107.

Tsukada, M., Schröder, M., Roos, T. C., Chandraratna, R. A., Reichert, U., Merk, H. F., Orfanos, C. E., & Zouboulis, C. C. (2000). 13-cis retinoic acid exerts its specific activity on human sebocytes through selective intracellular isomerization to all-trans retinoic acid and binding to retinoid acid receptors. *Journal of investigative dermatology*, *115*(2), 321-327.

Tüzün, Y., Keskin, S., & Kote, E. (2010). The role of Helicobacter pylori infection in skin diseases: Facts and controversies. *Clinics in dermatology*, *28*(5), 478-482.

Utaş, S., Özbakir, Ö., Turasan, A., & Utaş, C. (1999). Helicobacter pylori eradication treatment reduces the severity of rosacea. *Journal of the American Academy of Dermatology*, *40*(3), 433-435.

Valdez, G. F. de, De Giori, G. S., de Ruiz Holgado, A. P., & Oliver, G. (1985). Effect of drying medium on residual moisture content and viability of freeze-dried lactic acid bacteria. *Applied and Environmental Microbiology*, *49*(2), 413-415.

Van Der Meer, J. B., Glazenburg, E. J., Mulder, P. G., Eggink, H. F., & Coenraads, P. J. (1999). The management of moderate to severe atopic dermatitis in adults with topical fluticasone propionate. The Netherlands Adult Atopic DermatitisStudy Group. The British journal of dermatology, 140(6), 1114-1121.

Varani, J., Perone, P., Spahlinger, D. M., Singer, L. M., Diegel, K. L., Bobrowski, W. F., & Dunstan, R. (2007). Human skin in organ culture and human skin cells (keratinocytes and fibroblasts) in monolayer culture for assessment of chemically induced skin damage. *Toxicologic pathology*, *35*(5), 693-701.

Verhagen, J., Akdis, M., Traidl-Hoffmann, C., Schmid-Grendelmeier, P., Hijnen, D., Knol, E. F., ... & Akdis, C. A. (2006). Absence of T-regulatory cell expression and function in atopic dermatitis skin. *Journal of allergy and clinical immunology*, *117*(1), 176-183.

Verstraete, W., Landeghem, K., De Windt, W., Temmerman, R., (2007, June 18) Microbiological Results of PIP Healthcare Cleaning in a Clinical Environment. Retrieved December 1, 2014, from http://www.chrisal.com/uploads/1/1/0/8/11082217/_____02-university_and_hospital_report-belgium-june-07.pdf

Vos, W. M., Engstrand, L., Drago, L., Reid, G., Schauber, J., Hay, R., Mendling, W., Schaller, M., Spiller, R., Gahan, C. G. & Rowland, L. (2012). Human Microbiota in Health and Disease. *Selfcare 3*(S1), 1-68.

Vrese, M. & Schrezenmeir, J. (2008). Probiotics, Prebiotics and Synbiotics. *Adv Biochem Engin/ Biotechnol 111*, 1-66.

Wang, Y., Kuo, S., Shu, M., Yu, J., Huang, S., Dai, A., Richard, L. G, & Huang, C. M. (2014). Staphylococcus epidermidis in the human skin microbiome mediates fermentation to inhibit the growth of Propionibacterium acnes: implications of probiotics in acne vulgaris. *Applied microbiology and biotechnology*, *98*(1), 411-424.

Watson, W. C., Paton, E., & Murray, D. (1965). Small-bowel disease in rosacea. *The Lancet*, *286*(7402), 47-50.

Weinberg, J. M. (2005). The anti-inflammatory effects of tetracyclines. *Cutis; cutaneous medicine for the practitioner*, *75*(4 Suppl), 6-11.

Weiss, R. A., Weiss, M. A., & Beasley, K. L. (2002). Rejuvenation of photoaged skin: 5 years results with intense pulsed light of the face, neck, and chest. *Dermatologic surgery*, *28*(12), 1115-1119.

Wertz, P. W., Miethke, M. C., Long, S. A., Strauss, J. S., & Downing, D. T. (1985). The composition of the ceramides from human stratum corneum and from comedones. *Journal of investigative dermatology*, *84*(5), 410-412.

Whitehead, J. (2009). Intestinal alkaline phosphatase: The molecular link between rosacea and gastrointestinal disease?. *Medical hypotheses*, *73*(6), 1019-1022.

Whitehead, J. (2009). Intestinal alkaline phosphatase: The molecular link between rosacea and gastrointestinal disease?. *Medical hypotheses*, *73*(6), 1019-1022.

Widner, B., Behr, R., Von Dollen, S., Tang, M., Heu, T., Sloma, A., Sternberg, D., DeAngelis, P.L., Weigel, P.H., Brown S. (2005) Hyaluronic acid production in *Bacillus subtilis*. *Applied and Environmental Microbiology*, *71(7)*, 3747-3752.

Wilkin, J., (2010) "Oral Isotretinoin: The US Approach" *Pathogenesis and Treatment of Acne and Rosacea*. Ed. Zouboulis, C. C., Katsambas, A. D., & Kligman, A. M. *Springer* 472-475

Williams, H. C., Dellavalle, R. P., & Garner, S. (2012). Acne vulgaris. *The Lancet*, *379*(9813), 361-372.

Wilson, B.A., Ho, M. (2013). *Pasteurella multocida*: from Zoonosis to Cellular Microbiology. *Clinical Microbiology Reviews*, *26(3)*, 631-655.

Woessner, J. F. (1991). Matrix metalloproteinases and their inhibitors in connective tissue remmodeling. *The FASEB Journal*, *5*(8), 2145-2154.

Wollenberg, A., Sharma, S., von Bubnoff, D., Geiger, E., Haberstok, J., & Bieber, T. (2001). Topical tacrolimus (FK506) leads to profound phenotypic and functional alterations of epidermal antigen-presenting dendritic cells in atopic dermatitis. Journal of allergy and clinical immunology, 107(3), 519-525.

Wollina, U. (1996). Rhinophyma-unusual expression of simple-type keratins and S1 00A in sebocytes and abundance of VIP receptor-positive dermal cells. *Histol Histopathol*, 11, 111–115

Wollina, U. (2014). Recent advances in the understanding and management of rosacea. *F1000prime reports*, *6*.

XU, H. Y., Tian, W. H., Wan, C. X., Jia, L. J., Wang, L. Y., Yuan, J., Chun-Mei. L, Zeng. M., & Wei, H. (2008). Antagonistic potential against pathogenic microorganisms and hydrogen peroxide production of indigenous lactobacilli isolated from vagina of Chinese pregnant women. Biomedical and Environmental Sciences, 21(5), 365-371.

Yamamoto, A., Serizawa, S., Ito, M., & Sato, Y. (1991). Stratum corneum lipid abnormalities in atopic dermatitis. *Archives of dermatological research*, *283*(4), 219-223.

Yamasaki, K., & Gallo, R. L. (2009). The molecular pathology of rosacea. *Journal of dermatological science*, *55*(2), 77-81.

Yamasaki, K., & Gallo, R. L. (2011). Rosacea as a disease of cathelicidins and skin innate immunity. *Journal of Investigative Dermatology Symposium Proceedings, 15 (1),* 12-15. Nature Publishing Group

Yamasaki, K., Di Nardo, A., Bardan, A., Murakami, M., Ohtake, T., Coda, A., Dorschner, R. A., Bonnart, C., Descargues, P., Hovnanian, A., Morhenn, V. B., & Gallo, R. L. (2007). Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea. *Nature medicine*, *13*(8), 975-980.

Yamasaki, K., Kanada, K., Macleod, D. T., Borkowski, A. W., Morizane, S., Nakatsuji, T., Cogen, A. L., & Gallo, R. L. (2010). TLR2 expression is increased in rosacea and stimulates enhanced serine protease production by keratinocytes. *Journal of Investigative Dermatology*, *131*(3), 688-697.

Yang, Z., Yu, H., Cheng, B., Tang, W., Dong, Y., Xiao, C., & He, L. (2009). Relationship between the CAG repeat polymorphism in the androgen receptor gene and acne in the Han ethnic group. *Dermatology*, *218*(4), 302-306.

Ying, D. Y. & Demarchi, G. (2013). Probiotics and Prebiotics. In G. W. Smithers & M. A. Augustin (Ed.), *Advances in Dairy Ingredients* (269 - 276). Oxford, UK: Wiley - Blackwell.

Yosipovitch, G., Goon, A. T. J., Wee, J., Chan, Y. H., Zucker, I., & Goh, C. L. (2002). Itch characteristics in Chinese patients with atopic dermatitis using a new questionnaire for the assessment of pruritus. *International journal of dermatology*, *41*(4), 212-216.

Yosipovitch, G., Goon, A., Wee, J., Chan, Y. H., & Goh, C. L. (2000). The prevalence and clinical characteristics of pruritus among patients with extensive psoriasis. *British Journal of Dermatology*, *143*(5), 969-973.

Yosipovitch, G., Xiong, G. L., Haus, E., Sackett-Lundeen, L., Ashkenazi, I., & Maibach, H. I. (1998). Time-dependent variations of the skin barrier function in humans: transepidermal water loss, stratum corneum hydration, skin surface pH, and skin temperature. *Journal of investigative dermatology*, *110*(1), 20-24.

Yun, J. W., Seo, J. A., Jeong, Y. S., Bae, I. H., Jang, W. H., Lee, J., Lim, K. M., & Park, Y. H. (2011). TRPV1 antagonist can suppress the atopic dermatitis-like symptoms by accelerating skin barrier recovery. *Journal of dermatological science*, *62*(1), 8-15.

Zhang, G., & Ghosh, S. (2001). Toll-like receptor–mediated NF-κB activation: a phylogenetically conserved paradigm in innate immunity. *Journal of Clinical Investigation*, *107*(1), 13-19.

Zouboulis, C. C. (2004). The human skin as a hormone target and an endocrine gland. *HORMONES-ATHENS-*, *3*, 9-26.

Zouboulis, C. C., Korge, B., Akamatsu, H., Xia, L., Schiller, S., Gollnick, H., & Orfanos, C. E. (1991). Effects of 13-cis-retinoic acid, all-trans-retinoic acid, and acitretin on the proliferation, lipid synthesis and keratin expression of cultured human sebocytes in vitro. *Journal of investigative dermatology*, *96*(5), 792-797.

Zouboulis, C., Eady, A., Philpott, M., Goldsmith, L. A., Orfanos, C., Cunliffe, W. C., & Rosenfield, R. (2005). What is the pathogenesis of acne?. *Experimental dermatology*, *14*(2), 143-143.

Zouboulis, C.,C., (2010) 'Acne and Antimicrobial Lipids'' *Pathogenesis and Treatment of Acne and Rosacea*. Ed. Zouboulis, C. C., Katsambas, A. D., & Kligman, A. M. *Springer* 179-182.

APPENDIX

Keywords used in literature research

- Rosacea

Rosacea
Symptoms
Quality of life
Depression
Risk factors
UV light
Disease
Bacteria
Helicobacter pylori
Staphylococcus epidermidis
Demodex folliculorum
Psychological stress
Oxidative stress
ROS
SIBO
Cathelicidin
Neurotransmitters
Topical treatments
Oral treatments
Probiotics

- Acne

Acne
Acne vulgaris
Symptoms
Pathology
Therapy
Patients life
Cutaneous condition
Comedogenesis
Pilocebaceous unit remodelling
Sebum seborrhea
Sebaceous gland lipid
Squalene peroxidation
Propionibacterium acnes
Hyperkeratinization
Inflammation
Neuropeptide
Peptidase
Substance P
Antibiotic
Retinoic acid
Benzoyl peroxide
Isotretinion
Hormonal treatment
Staphylococcus epidermidis

- Atopic dermatitis

Atopic dermatitis
Pathophysiology
Treatments
Genetic AD
Environmental AD
Symptoms
Skinbarrier
Filaggrin
Ceramides
TRPV1
Immune reaction
Itching
Pruritic properties
Sleep itching
Stress
Quality of life
Staphylcoccus Aureus

- Healthy skin & skin microbiome

Skin
Microbiome
Microbiota
Bacteria
Fungi
Composition

- Pre-, post- and probiotics

Health
Treatment

Lactobacillus
Bifidobacteria
Human
Review
Gut
Prebiotics
Postbiotics
Probiotics
Microbiota
Microbiome
Intestine
Strains

- Probiotics (market study)

Probiotics
Skin
Treatment
Clinical study

Human
Bacteria
See also skin conditions

- Market study

Probiotics
Cream
Skin treatment
Healthy skin
Face
Beauty
Products
Mask
Skin care
Treatment