# The role of lactobacilli in inhibiting skin pathogens

# The role of lactobacilli in inhibiting skin pathogens

Lize Delanghe [1,2] *, Irina Spacova [1] *, Joke Van Malderen [1], Eline Oerlemans [1], Ingmar Claes [1,2], Sarah Lebeer[1]

*Equal contribution

[1] University of Antwerp, Department of Bioscience Engineering, Groenenborgerlaan 171, B-2020 Antwerp, Belgium.

[2] YUN NV, Galileilaan 15, B-2845 Niel, Belgium.

**Correspondence**: Sarah Lebeer, University of Antwerp, Department of Bioscience Engineering, Groenenborgerlaan 171, B-2020 Antwerp, Belgium. Telephone: +3232653285 E-mail: sarah.lebeer@uantwerpen.be

## Abstract

The human skin microbiota forms a key barrier against skin pathogens and is important in modulating immune responses. Recent studies identify lactobacilli as endogenous inhabitants of healthy skin, while inflammatory skin conditions are often associated with a disturbed skin microbiome. Consequently, lactobacilli-based probiotics are explored as a novel treatment of inflammatory skin conditions through their topical skin application. This review focuses on the potential beneficial role of lactobacilli (family *Lactobacillaceae*) in the skin habitat, where they can exert multifactorial local mechanisms of action against pathogens and inflammation. On one hand, lactobacilli have been shown to directly compete with skin pathogens through adhesion inhibition, production of antimicrobial metabolites, and by influencing pathogen metabolism. The competitive anti-pathogenic action of lactobacilli has already been described mechanistically for common different skin pathogens, such as *Staphylococcus aureus*, *Cutibacterium acnes*, and *Candida albicans*. On the other hand, lactobacilli also have an immunomodulatory capacity associated with a reduction in excessive skin inflammation. Their influence on the immune system is mediated by bacterial metabolites and cell wall-associated or excreted microbe-associated molecular patterns (MAMPs). In addition, lactobacilli can also enhance

the skin barrier function, which is often disrupted as a result of infection or in inflammatory skin diseases. Some clinical trials have already translated these mechanistic insights into beneficial clinical outcomes, showing that topically applied lactobacilli can temporarily colonize the skin and promote skin health, but more and larger clinical trials are required to generate *in vivo* mechanistic insights and in-depth skin microbiome analysis.

**Introduction**

The skin is considered one of the largest organs in the human body (1) which plays an important barrier and protective role against invasion of pathogens, foreign substances, ultraviolet (UV) radiation, moisture loss and body temperature fluctuations (1). It is simultaneously influenced by various exogenous (surface interactions) and endogenous factors, such as systemic interactions and the skin metabolome, and forms the primary interface between the external environment and the internal body. The skin consists of two main layers, the top epidermis and dermis, and a third layer, the hypodermis or the subcutaneous tissue. The epidermis is the outermost protective layer and is formed by multiple layers of differentiated keratinocytes, with a top layer of terminally differentiated keratinocytes (stratum corneum) (2).

The skin surface is colonized by diverse microbial communities, which collectively form the skin microbiota and play an important role in antagonizing skin pathogens and modulating immune responses. For example, skin commensals mediate the production of antimicrobial peptides and prevent hypersensitivity to allergens by promoting type 1 T-helper (Th1) cells (3,4). Despite the large number of skin microbiome studies, the activity and function of many skin microbes is still underexplored. Recently, lactobacilli have emerged as skin microbiota members associated with health. Different taxa of the *Lactobacillaceae* family (see (5) for the updated taxonomy) have been shown to be decreased on the skin of patients with skin disorders, such as diaper dermatitis (6), atopic dermatitis (7) and acne vulgaris (8). Therefore, their topical application in the treatment of common skin conditions through interventions in the skin microbiota show significant potential, as we demonstrated for acne vulgaris (8), but it should be further explored.

Beneficial lactobacilli have a long history of use to improve human health, for example as probiotics, i.e. “live micro-organisms that, when applied in adequate amounts, promote a health effect on the host” (9) or postbiotics, i.e. “preparations of inanimate microorganisms and/or their components that confer a health benefit on the host” (Salminen *et al*., submitted). Traditional orally administered probiotic lactobacilli can indirectly promote skin health (10), for example through immunomodulation, resulting in effects such as reduced skin reactivity and transepidermal water loss (11). These effects are facilitated via the gut-skin axis and can be linked with direct interaction of lactobacilli with the gut immune system, production of bioactive molecules (e.g. acetate) or gut microbiome modulation (10). Indeed, meta-analyses have shown that oral products with specific strains of lactobacilli are effective for the treatment of inflammatory skin conditions (12,13), such as atopic dermatitis, acne vulgaris and seborrheic dermatitis, and can improve wound healing (13,14).

Recently, topical application of lactobacilli is emerging as a potentially more efficient and direct strategy for the prevention and treatment of skin disorders, especially since it has been suggested that protective immunity against skin pathogens rather depends on the bacteria found on the skin than the gut microbiota (4). In this review, we will focus on the potential beneficial role of lactobacilli on the skin.

**Factors shaping the seeding and maturation of the skin microbiota**

During and after birth, the skin of neonates is colonized by microorganisms originating from the mother’s birth canal, the outside environment, and the exposure to other individuals. Various studies have shown that the mode of delivery influences the composition of the neonates' skin microbiome during this neonatal window of opportunity crucial for immune homeostasis (15,16). For example, *16S rRNA* amplicon sequencing of a cohort of neonates (n=81; born by vaginal delivery or Cesarean section-rate cesarean section 33.1%) has shown that the skin of newborns contains the highest relative abundance of *Lactobacillaceae* spp. of all body sites sampled at the day of their birth: 25.9% relative abundance (+/- 30.2% standard deviation) compared to respectively 16.6% for nares (+/- 26.6%), 15.4% for oral (+/- 19.0%) and 4.4% for stool (+/- 17.4%) samples. In this cohort, vaginally delivered babies also showed a significantly higher abundance of skin lactobacilli compared to neonates born by

(unlabored) cesarean delivery (16). The latter group also showed a significant reduced alpha-diversity in skin microbiome (16), supporting an earlier study which illustrated that neonates born through cesarean section showed reduced microbial diversity on their skin, oral and gut microbiome (15). The skin microbiome of neonates born by caesarian section (as compared to vaginal birth) appeared to resemble more the microbiota of the mother's skin (beta-diversity analysis), dominated by *Staphylococcus* spp., *Cutibacterium* spp. and *Corynebacterium* spp. (16,17). Currently, shotgun metagenomic studies of the neonate skin microbiome are lacking (16), hindering classification of the neonate skin microbiota at sub-genus level. However, as it was suggested that the mother's vaginal and/or skin microbial community is vertically transmitted to the baby, we can expect that the transferred lactobacilli belong to typically vaginally associated taxa, such as *Lactobacillus crispatus, Lactobacillus iners, Lactobacillus gasseri* and *Lactobacillus jensenii*, similarly as was found for neonate oral and gut microbiomes (16–19). However, it should also be stressed that the neonate's microbiota is still undifferentiated at the skin and other body habitats (17). After birth, it undergoes rapid changes influenced by the physiological characteristics of the skin and by multiple interactions with the environment. Already after the first week of life, the skin microbiome changes towards a more adult-like composition. At 6 weeks, the relative abundance of lactobacilli on the skin has been shown to drop to 0.3% (+/-0.7% standard deviation) (16).

The skin has different physiological characteristics across body sites, such as pH, dryness (production of sebum and sweat), body temperature and morphology, which all provide different microenvironments and influence skin microbiome composition (20–22). For example, the surface of the skin of healthy individuals has a low pH of approximately 4 to 6 (22,23), representing a key component for the maintenance of the epidermal barrier function and being an important defense mechanism against pathogens (24,25). The production of sebum and sweat affect the skin microbiota respectively by providing an antibacterial shield and by the thermoregulation of the skin by the release of heat from evaporation of water (20,26). Following studies focusing on single skin sites (27–29), Grice *et al*. (21) and Costello et al. (30) studied the microbiome of various skin body sites and reported that the four most detected phyla were *Actinobacteria* (51.8%), *Firmicutes* (24.4%), *Proteobacteria* (16.5%),

and *Bacteroidetes* (6.3%) (21). More than 62% of the detected *16S rRNA* sequences were associated with three genera: *Corynebacteria* (22.8%- dominates moist body sites such as nostril and inner elbow and dry microenvironments such as the inside of the mid-forearm), *Propionibacteria* (23.0%- most abundant at sebaceous site such as scalp, face, back and inside the ear) and *Staphylococcus* (16.8%- also dominant at moist body sites)(21). *Proteobacteria* were also commonly found at moist and dry microenvironments, while *Flavobacteriales* were also typically found at the latter. Most adult skin microbiome studies also rely on amplicon sequencing strategies (reviewed in (2)) limited in their taxonomic resolution, but some metagenomic studies have brought clarity to the species-level composition of the skin microbiome. *Cutibacterium acnes* and *Staphylococcus epidermidis* are overall the most dominant species on healthy adult skin (2,31,32).

While the human microbiota changes over time and is subjected to multiple influencing factors, we have recently found, by *16S rRNA* amplicon sequencing and mining of publicly available skin metagenome shotgun datasets (by using the curatedMetagenomicData R-package), that lactobacilli are still prevalent on the adult skin. Species typically associated with the human vagina (*L. crispatus, L. iners, L. gasseri and L. jensenii*) are still the most prevalent lactobacilli on the adult skin (8). Also, members of the more niche-flexible *Lactobacillaceae* taxa, from the *Lactiplantibacillus* and *Lactocaseibacillu*s, were frequently detected. Although lactobacilli generally do not occur in high relative abundances in adults (8,31), they can still be regarded as endogenous minor members of the skin microbiota with potential beneficial functions as we will review below.

**Disturbances of the skin microbiota in skin disorders**

While there is no definition of a healthy skin microbiota, a link has been proposed between the skin microbiota taxonomic composition and diversity, and general skin health (2). Certain taxa of the skin microbiota are considered opportunistic pathogens that can cause disease in susceptible individuals, including common opportunistic pathogens *Staphylococcus aureus, C. acnes* and *Streptococcus pyogenes. S. aureus* is associated with infectious skin diseases such as impetigo (33,34), folliculitis (35), furunculosis, hidradenitis suppurativa (36), psoriasis (37), abscesses (38) and even acne vulgaris (39). *C. acnes* is traditionally mainly linked to acne vulgaris, a chronic inflammatory skin condition of the

sebaceous follicles and glands (40,41). *Streptococcus pyogenes* is found on burn wounds (42) and associated with skin conditions such as impetigo, erysipelas and cellulitis (43). Also the fungal pathogen *Candida albicans* causes skin infections (44) and is associated with skin conditions such as atopic dermatitis (45). Skin infections caused by these pathogens can result in excessive inflammation (44,46), skin barrier disruption (47,48) and a decrease in skin microbiome diversity (Figure 1), which can manifest as such or are linked to skin conditions such as psoriasis and atopic dermatitis (49–51).

In addition to the microbiota composition, the skin microbiota diversity is also linked to human health. As stated by the hygiene (52), old friends and biodiversity (53) hypotheses, microbial exposure in early childhood is linked to activation of immune regulatory pathways and reduction in overactive immune responses associated with immune-mediated conditions, such as allergic and inflammatory diseases (54,55). For example, attendance of day care in the first years of life and growing up or living in a farm environment has a positive effect on skin microbiome diversity (55,56). While these hypotheses are mainly based on epidemiological associations, recent experimental studies are investigating the positive influence of the environment on the skin health of children. Roslund *et al*. (55) have recently found that enriching daycare center yards for microbial biodiversity was associated with diversification of skin *Gammaproteobacteria* in children and elevated levels of systemic regulatory T cells and transforming growth factor-β1 (TGF-β1) responsible for regulation of inflammatory processes. The ratio of the regulatory cytokine interleukin-10 (IL-10) to pro-inflammatory IL-17A was increased. These promising data indicate that the microbiome and immune system of children can be beneficially influenced by the exposure to different environmental bacterial communities (55). Similar research is currently ongoing in the context of the large-scale BELSPO B@SEBALL (Biodiversity at School Environments Benefits for ALL) Belgian study that aims to unravel how the school playground environment positively affects children's immune and mental health through changes in the skin microbiome (57).

Indeed, in various skin disorders, such as atopic dermatitis and acne vulgaris, the skin microbiota appears to be less diverse and more dominated by opportunistic pathogens in comparison with healthy individuals. In patients with atopic dermatitis, a reduction in the overall microbial diversity has been

observed and flare-ups of the disease are associated with an abundant colonization of *Staphylococcus* spp. Meta-analysis (11 human studies, *16S rRNA* amplicon sequencing) and more recent metagenomic shotgun and transcriptome analysis detected a lower bacterial diversity on atopic dermatitis skin, more specific a reduction in *C. acnes, Lactobacillus* spp*., Burkholderia* spp*., Acinetobacter* spp*., Corynebacterium* spp*.,* and *Enhydrobacter* spp*.* (7,51). An association was also found between the disease severity and reduced Shannon diversity (49). In addition, *S. aureus* was shown to be significantly increased during flares: in 70% of atopic dermatitis patients the affected skin was colonized by *S. aureus*, in 39% of atopic dermatitis patients *S. aureus* is also found on unaffected skin (48). Increased *S. aureus* abundance was even correlated with an increased SCORAD (Scoring Atopic dermatitis) for disease severity (59). A reduced microbial diversity is thought to function in combination with the locally disturbed immune system in atopic dermatitis patients to lower the inhibition of *S. aureus* (25). It is well-known is that *S. aureus* has a pronounced pro-inflammatory effect on the skin due to toxins, modulins, superantigens and proteases produced by this species (24). This inflammatory effect of *S. aureus* on the skin has been shown to be strain dependent, with severe atopic dermatitis patients apparently being colonized with a single clade of *S. aureus* during disease flares (59). Moreover, *S. aureus* demonstrates strain-specific differences in eliciting skin inflammation, epidermal thickening and atopic dermatitis-specific immune signatures (59–61). This has also been linked to virulence factors, as another study showed that atopic dermatitis strains of *S. aureus* appeared to secrete higher amounts of toxins, such as toxic shock syndrome toxin 1 (TSST-1), staphylococcal enterotoxin B (SEB) and staphylococcal enterotoxin C (SEC) (62). In a recent study, enrichment of bacterial toxins, glycolysis, tryptophan metabolism and genes for inflammatory signaling was also detected in atopic dermatitis skin samples with a high abundance of *S. aureus* (51).

A second highly prevalent skin condition that is associated with the skin microbiota, is acne vulgaris. As previously mentioned, acne vulgaris is a chronic inflammatory skin condition of the sebaceous follicles and glands linked to a high prevalence of *C. acnes*, in addition to hormonal factors (41). *C. acnes* is primarily known as a skin commensal in both acne patients and healthy individuals: it helps maintain a low skin pH by releasing fatty acids and inhibiting pathogens such as *S. aureus* and

187 *Streptococcus* (21,63). In acne vulgaris, *C. acnes* is known to promote the inflammation of the skin,
188 especially by secreting lipase enzymes that metabolize sebum into free fatty acids (8,64). Recent
189 studies suggest that the pathogenesis characteristics of *C. acnes* are also strain dependent. Acne-
190 related strains show an enrichment in virulence factors, an increased production of porphyrin, which
191 can generate reactive oxygen species (ROS) and induce inflammation, and have a reduced abundance
192 of metabolic synthesis genes (32,41,65). Next to *C. acnes,* also other skin bacteria are often mentioned
193 to play a role in the acne pathogenesis. For example, *Staphylococcus* species can be linked to acne
194 pathogenesis as pathobionts or disease modulators (39). Yet, the strain-dependency for *S. aureus* in
195 acne pathogenesis is not well studied.
196 In addition to atopic dermatitis and acne, a wide range of other skin diseases could also be linked to a
197 disturbed skin microbiome and a high colonization with pathobionts, as mentioned above, such as
198 psoriasis (37), folliculitis decalvans (35), impetigo (33) and hidradenitis suppurativa (36).

199 **Strain-specific mechanisms of lactobacilli against skin infections**

200 Various mechanisms of action could underlie beneficial actions of lactobacilli applied in the skin niche
201 against bacterial infection and the related disease symptoms (8,66). They include both probiotic factors
202 that can directly mediate health benefits (Figure 1) and adaptation factors that allow lactobacilli to
203 temporarily colonize the skin. Of note, not only live lactobacilli, but also their postbiotic formulations
204 in the form of heat-inactivated bacteria or lysates containing various microbe-associated molecular
205 patterns (MAMPs) and bioactive metabolites have been shown to result in beneficial effects *in vitro*
206 (67,68) and *in vivo* (69). Interestingly, bacteria and their products can be detected at 16S rRNA gene
207 level and by immunostaining also in the skin layers below the epidermis, suggesting that their influence
208 can extend to the dermis and possibly beyond (70).

209 Exogenously applied lactobacilli, such as *L. rhamnosus* GG (67,71) and *Limosilactibacillus reuteri* DSM
210 17938 (72) have been shown to exert direct and indirect anti-pathogenic action. Through direct binding
211 interactions, lactobacilli or their lysates can prevent the adhesion of pathogens to keratinocytes, or
212 actively displace the adhered pathogens, as demonstrated *in vitro* against *S. aureus* (67). For *L.*
213 *rhamnosus* GG these effects appear to be at least in part mediated by its high adherence to human

keratinocytes through the unique SpaCBA pili (Figure 1) (71). Such high adhesion can also be an important adaptation factor for a longer retention time on the skin, and possibly also for co-aggregation with *S. aureus* (71). Of note, a closely related strain *L. rhamnosus* GR-1 was not able to prevent the adhesion of *S. aureus* to keratinocytes, highlighting that the anti-pathogenic action of lactobacilli is often strain-specific (71).

Lactobacilli are also a source of potent antimicrobial metabolites, including L- and D-lactic acid that are known to inhibit pathogen growth (71) and promote a healthy acidic pH of the skin (73) (Figure 1). Other cell-cell interactions and metabolites of lactobacilli can also impact skin pathogens that are more resistant to lower pH, including the fungal pathogen *C. albicans* (74). Our group identified exopolysaccharides and the major peptidoglycan hydrolase Msp1 as key molecules of *L. rhamnosus* GG that inhibited *C. albicans* hyphae formation and adhesion to host epithelial cells (75,76). More specifcially, the Msp1 enzyme can function as a chitinase capable of breaking down chitin important for fungal hyphae formation. In addition, lactic acid bacteria can produce bacteriocin-like substances or bacteriocins, i.e. ribosomally synthesized antimicrobial peptides, active against opportunistic skin pathogens such a *C. acnes* (77), *S. pyogenes* and *S. aureus* (78). Finally, lactobacilli can influence pathogen metabolism linked to disease symptoms. As such, the lipase activity of *C. acnes* which helps metabolize sebum into free fatty acids contributing to acne (64) was significantly reduced under the influence of *L. rhamnosus* GG, *L. plantarum* WCFS1 and *L. pentosus* KCA1 (8).

Specific strains to lactobacilli also have an immunomodulatory function and can reduce excessive skin inflammation. These effects can be mediated by bacterial metabolites (e.g. Msp2/p40 of *L. rhamnosus* GG) or cell wall-associated and excreted MAMPs (e.g. SpaCBA pili of *L. rhamnosus* GG; Figure 1) that are recognized by the pattern-recognition receptors on host cells, such as Toll-like receptors (TLRs) (71,79,80). In contrast to pathogenic immune interactions, the resulting immune response rather leads to balanced immunity characterized by a reduction in pro-inflammatory signaling in favor of regulatory pathways (79). For example, interleukin IL-6 and IL-8 were both reduced upon application of live and lysed *L reuteri* DSM 17938 in reconstructed human epidermis and native skin models of UV B radiation-induced inflammation (72). Application of extracellular vesicles of *L. plantarum* APsulloc 331261 was

241 also associated with induction of the regulatory cytokine IL-10, macrophage-characteristic cytokines
242 IL-1β and granulocyte-macrophage colony-stimulating factor (GM-CSF), inhibition of pro-inflammatory
243 cell surface markers (e.g. Human Leukocyte Antigen – DR α (HLA-DRα) of M1 macrophages) and
244 promotion of anti-inflammatory M2 macrophages *in vitro* (81). While insights from oral application of
245 lactobacilli in skin disease suggest beneficial induction of IL-10 and CD4+CD25+Foxp3+ regulatory T
246 (Treg) lymphocytes (as reviewed in (10)), stimulation of Treg cells by cutaneous application of
247 lactobacilli or their metabolites requires additional mechanistic research. Simultaneous co-incubation
248 of human THP-1 monocytes with *L. rhamnosus* GG and *S. aureus* resulted in a less pronounced
249 induction of nuclear factor (NF)-κB compared to their co-incubation with *S. aureus* alone, although it
250 was challenging to disentangle the immunomodulatory and anti-pathogenic effects of lactobacilli in
251 this *in vitro* experimental set-up (81). In addition to direct stimulation of immune and epithelial cells,
252 antimicrobial compounds produced by lactobacilli could help suppress opportunistic skin pathogens
253 such as *S. aureus* (8), similarly to the antimicrobial compounds produced by the host (3). In turn, a
254 balanced microbiota consisting of commensals such as *S. epidermidis* in contrast to *S. aureus* could
255 help promote Treg activity and release of anti-inflammatory IL-10 (83).
256 Cutaneous application of specific lactobacilli is also associated with enhancement of the skin barrier
257 function, which is key for preventing and treating a wide range of skin conditions associated with
258 barrier disruption, such as atopic dermatitis (84). As such, *L. plantarum* ATCC 10241, *L. reuteri* ATCC
259 55730 or *L. rhamnosus* GG have been shown to enhance epithelial barrier function through increasing
260 expression of tight-junction proteins in primary human keratinocytes (85). In a reconstructed human
261 epidermis model the barrier-enhancing mechanisms of lactobacilli were strain-specific. As such, the
262 key proteins stimulated by *L. rhamnosus* GG were shown to be claudin-1 and occludin (86), while live
263 and lysed *L. reuteri* DSM 17938 enhanced aquaporin 3 (AQP3) and laminin A/B levels, respectively (72).
264 Furthermore, the lysate of *L. rhamnosus* GG could increase re-epithelialization of keratinocytes by
265 promoting their migration (68). While the exact effector molecules in the skin niche are yet to be
266 determined, the secreted Msp2/p40 protein of *L. rhamnosus* GG (Figure 1) has previously been shown
267 to maintain barrier function in the colon epithelium by interacting with the epidermal growth factor

receptor (EGRF) (80). This receptor is also widely present on skin cells such as keratinocytes and is involved in skin repair and inflammation modulation (87).

**Promising intervention studies with topical probiotic lactobacilli**

The novel microbiome-level and mechanistic insights into the role of lactobacilli in the skin niche reviewed above, as well as tailored formulations for their application to the desiccated nutrient-poor environment of the skin (8), have paved the way for topical cutaneous application of exogenous lactobacilli. These insights can at least in part be translated into beneficial clinical outcomes. For example, in a recent translational study, our group demonstrated the feasibility and efficacy of applying a topical cream with live *L. rhamnosus* GG, *Lactiplantibacillus plantarum* WCFS1 and *Lactiplantibacillus pentosus* KCA1 in patients with mild-to-moderate acne symptoms, resulting in reduction in inflammatory lesions and comedone formation (8). Another study demonstrated local clinical improvement (SCORAD) with a lotion containing heat-treated *Lactobacillus johnsonii* NCC 533 in atopic dermatitis patients (69). Both skin treatments resulted in a reduction in relative abundance of staphylococci within the skin microbiome. In our study (8) the anti-inflammatory effects of exogenous lactobacilli application was still observed 4 weeks after discontinuation of the topical application in acne patients (8), pointing at an immunological mechanism. Considering the strain-specific activity of different lactobacilli and the various host targets through which they can exert their beneficial action, one of the advantages of applying mixtures of lactobacilli as in (8) could be their cumulative multifactorial working. Topical lactobacilli are also promising in clinical application for wound healing. *L. plantarum* ATC 10 has been shown to promote granulation, healing and decreased the bacterial load in burn wounds (88) and chronic venous ulcers in humans (89). In the latter study, this was associated with a reduction in apoptotic and necrotic cells and modulated IL-8 production (89).

In addition to lactobacilli, probiotics from other taxa are emerging and explored in clinical studies. Of note, besides lactobacilli, topical application of live *Roseomonas mucosa* (90) and *Vitreoscilla filiformis* lysate (91) have shown clinical improvement for atopic dermatitis. Furthermore, research with non-pathogenic *S. epidermidis* and *Staphylococcus hominis* strains hold promise for microbiome-based

therapies for atopic dermatitis through decreasing *S. aureus* colonization on human skin (92). Because of species- and strain-specific activity, inter-study comparison of alternative microbiome-based solutions is however difficult.

## Conclusions

Lactobacilli are endogenous inhabitants of healthy skin and carefully selected topical *Lactobacillaceae*-based probiotics are promising as an efficient and direct treatment of a wide range of skin conditions. At various skin sites, specific lactobacilli could demonstrate multifactorial local mechanisms of action against pathogens and inflammation. This includes direct anti-pathogenic activity, immunomodulation, promotion of skin barrier function, maintenance of a balanced microbiota and possibly additional mechanisms described in other body niches but yet to be demonstrated on the skin. Ultimately, *in vitro* mechanistic insights need to be translated into *in vivo* beneficial clinical outcomes. Clinical trials with topical lactobacilli formulations for different skin conditions have already shown promising results, but there is still a lack of sufficient large-scale trials, *in vivo* mechanistic insights and detailed skin microbiome analysis.

## Perspectives

- A wide range of skin conditions are linked with microbiome perturbations. Lactobacilli are promising as a topical microbial therapy against many of these conditions and their importance is becoming more evident as more skin microbiome studies are conducted.
- While much research still focuses on the oral administration of lactobacilli, the demonstrated multifactorial action of specific lactobacilli directly in the skin niche already includes anti-pathogenic activity, immunomodulation, promotion of barrier function and maintenance of a balanced microbiota. Considering their long history of use and safety, formulations with live lactobacilli have a high translational potential from *in vitro* directly to application in humans.
- An increasing number of clinical studies with topical lactobacilli are underway against common diseases such as atopic dermatitis. Larger double-blind placebo-controlled studies must be

encouraged. There is also a need for an integrated and more detailed analyses on skin microbiota modulation and strain-specific mechanisms of action in the skin niche.

**Abbreviations**

| | |
|---|---|
| TGF-β1 | transforming growth factor beta 1 |
| Treg | regulatory T cells |
| IL | interleukin |
| TSST-1 | toxic shock syndrome toxin 1 |
| SEB | Staphylococcal enterotoxin B |
| SEC | Staphylococcal enterotoxin C |
| MAMPs | microbe-associated molecular patterns |
| AMPs | antimicrobial peptides |
| Th | T helper cells |
| PRRs | pattern-recognition receptors |
| LLA | L-lactic acid |
| DLA | D-lactic acid |
| EPS | exopolysaccharides |
| Msp | major secreted protein |
| TLRs | toll-like receptors |
| GM-CSF | granulocyte-macrophage colony-stimulating factor |
| NF-κB | nuclear factor kappa B |

**Declaration of Interests**

I.S., J.V.M., and E.O. declare no conflicts of interest. L.D. and I.C. have a double affiliation: they are working at the R&D department of YUN NV, a Belgian biotech company (www.yun.be), originating from their research at the University of Antwerp. They are both still affiliated to UAntwerp and the research group of S.L for academic research and teaching tasks. S.L. was involved in the start-up of Yun and acts as a consultant in the scientific advisory board of Yun N.V. This review was written upon invitation. S.L. currently holds a grant from the European Research Council grant (Lacto-be 26850) in which the ecology, evolutionary history and beneficial potential of lactobacilli in multiple habitats is explored at fundamental level.

**Funding**

I.S. was supported by the IOF-POC University of Antwerp funding ReLACT. S.L. was supported by the European Research Council grant (Lacto-be 26850).

**Author contributions**

SL and IC drafted the outline of the review. LD, IS, EO and JVM wrote different parts of the paper. IS designed the figure. All authors proofread and approved the manuscript.

**Figure**

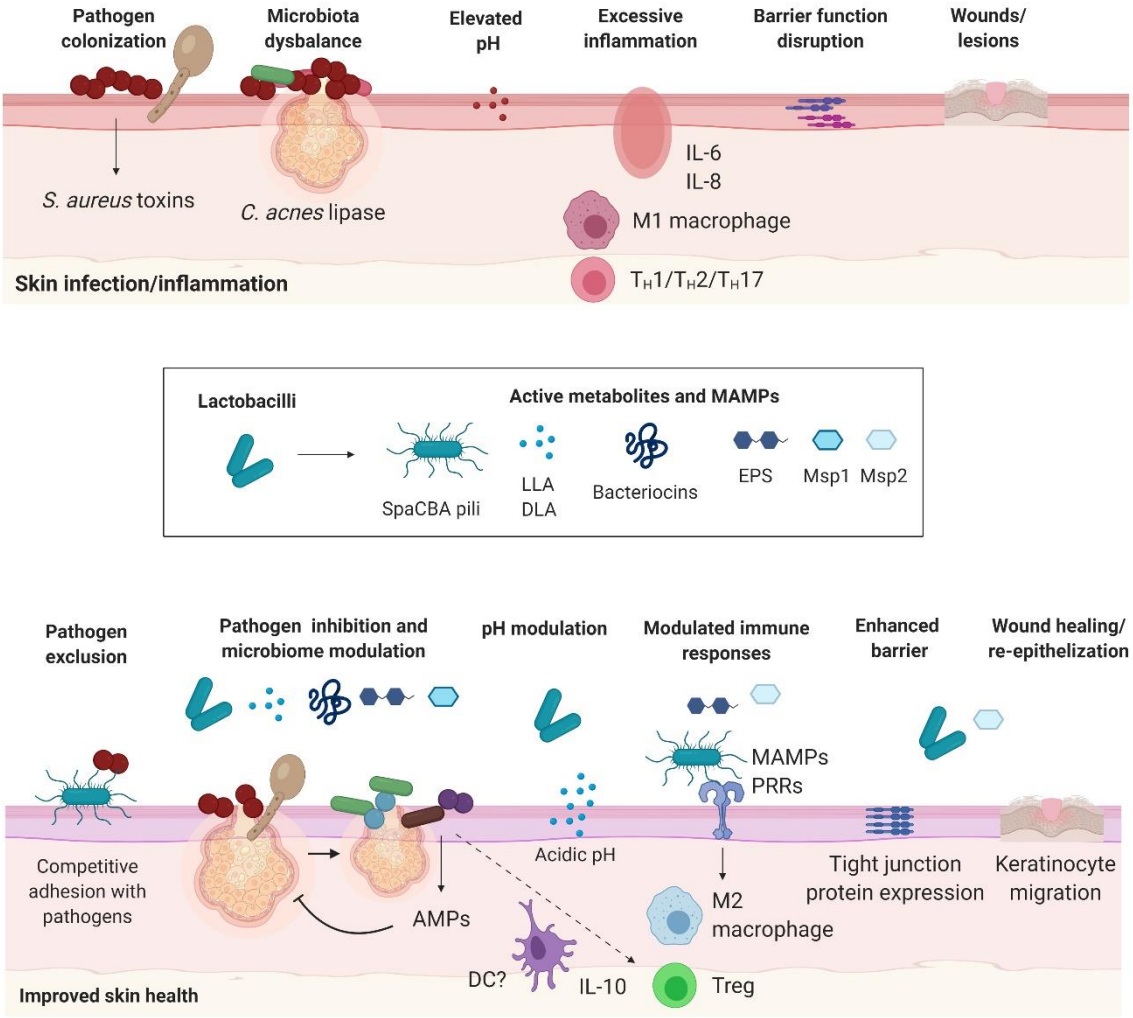


**Figure 1. Postulated strain-specific mechanisms of action resulting in beneficial effects of lactobacilli in the skin niche, including proposed probiotic molecules with effects demonstrated in the skin and/or other niches.** A textbook example combining several modes of action in the skin niche is the widely studied probiotic strain *L. rhamnosus* GG that can protect human keratinocytes from the pathogen *S. aureus* through direct inhibition of pathogen growth(8,71), as well as competitive binding and reduction in *S. aureus* adhesion(67) and interactions with TLR receptors on host cells(71). Abbreviations: AMPs: antimicrobial peptides; DC: dendritic cells; Th1, Th2, Th17: T helper cells; IL-17, IL-10: interleukins 17 and 10; MAMPs: microbe-associated molecular patters; PRRs: pattern-recognition receptors; LLA, DLA: L- and D-Lactic acid; EPS: exopolysaccharides; Msp1/2: major secreted proteins 1 and 2. Created with BioRender.com.

**References**


1. Gallo RL. Human Skin Is the Largest Epithelial Surface for Interaction with Microbes. Vol. 137, Journal of Investigative Dermatology. Elsevier B.V.; 2017. p. 1213–4.

2. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. Nat Rev Microbiol. 2018 Jan 15;16(3):143–55.

3. Ong PY, Ohtake T, Brandt C, Strickland I, Boguniewicz M, Ganz T, et al. Endogenous Antimicrobial Peptides and Skin Infections in Atopic Dermatitis. N Engl J Med. 2002 Oct 10;347(15):1151–60.

4. Naik S, Bouladoux N, Wilhelm C, Molloy MJ, Salcedo R, Kastenmuller W, et al. Compartmentalized control of skin immunity by resident commensals. Science (80- ). 2012 Aug 31;337(6098):1115–9.

5. Zheng J, Wittouck S, Salvetti E, Franz CMAP, Harris HMB, Mattarelli P, et al. A taxonomic note on the genus Lactobacillus: Description of 23 novel genera, emended description of the genus Lactobacillus Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. Int J Syst Evol Microbiol. 2020;

6. Zheng Y, Wang Q, Ma L, Chen Y, Gao Y, Zhang G, et al. Shifts in the skin microbiome associated with diaper dermatitis and emollient treatment amongst infants and toddlers in China. Exp Dermatol. 2019 Nov 16;28(11):1289–97.

7. Bjerre RD, Bandier J, Skov L, Engstrand L, Johansen JD. The role of the skin microbiome in atopic dermatitis: a systematic review. Br J Dermatol. 2017;177(5):1272–8.

8. Lebeer S, Oerlemans E, Claes I, Wuyts S, Henkens T, Spacova I, et al. Topical cream with live lactobacilli modulates the skin microbiome and reduce acne symptoms. bioRxiv. 2018 Nov 19;1–28.

9. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014 Aug 10;11(8):506–14.

10. O'Neill CA, Monteleone G, McLaughlin JT, Paus R. The gut-skin axis in health and disease: A paradigm with therapeutic implications. BioEssays. 2016 Nov 1;38(11):1167–76.

11. Gueniche A, Philippe D, Bastien P, Reuteler G, Blum S, Castiel-Higounenc I, et al. Randomised double-blind placebo-controlled study of the effect of Lactobacillus paracasei NCC 2461 on skin reactivity. Benef Microbes. 2014;5(2):137–45.

12. Panduru M, Panduru NM, Sələvəstru CM, Tiplica GS. Probiotics and primary prevention of atopic dermatitis: A meta-analysis of randomized controlled studies. J Eur Acad Dermatology Venereol. 2015 Feb 1;29(2):232–42.

13. Yu Y, Dunaway S, Champer J, Kim J, Alikhan A. Changing our microbiome: probiotics in dermatology. Br J Dermatol. 2019 Jul 28;182(1):bjd.18088.

14. El-Ghazely MH, Mahmoud WH, Atia MA, Eldip EM. Effect of probiotic administration in the therapy of pediatric thermal burn. Ann Burns Fire Disasters. 2016 Dec 31;29(4):268–72.

15. Biasucci G, Benenati B, Morelli L, Bessi E, Boehm G. Cesarean delivery may affect the early biodiversity of intestinal bacteria. J Nutr. 2008;138(9):1796–800.

16. Chu DM, Ma J, Prince AL, Antony KM, Seferovic MD, Aagaard KM. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. Nat Med. 2017;23(3):314–26.

17. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A. 2010 Jun 29;107(26):11971–5.

18. Ferretti P, Pasolli E, Tett A, Asnicar F, Gorfer V, Fedi S, et al. Mother-to-Infant Microbial Transmission from Different Body Sites Shapes the Developing Infant Gut Microbiome. Cell Host Microbe. 2018;

19. Dominguez-Bello MG, De Jesus-Laboy KM, Shen N, Cox LM, Amir A, Gonzalez A, et al. Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. Nat Med. 2016 Mar;22(3):250–3.

20. Grice EA, Segre JA. The skin microbiome. Nat Rev Microbiol. 2013;9(4):244–53.

21. Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, et al. Topographical and temporal diversity of the human skin microbiome. Science (80- ). 2009 May 29;324(5931):1190–2.

22. Dréno B, Araviiskaia E, Berardesca E, Gontijo G, Sanchez Viera M, Xiang LF, et al. Microbiome in healthy skin, update for dermatologists. J Eur Acad Dermatol Venereol. 2016 Dec;30(12):2038–47.

23. Lambers H, Piessens S, Bloem A, Pronk H, Finkel P. Natural skin surface pH is on average below 5, which is beneficial for its resident flora. Int J Cosmet Sci. 2006 Oct 1;28(5):359–70.

24. Geoghegan JA, Irvine AD, Foster TJ. Staphylococcus aureus and Atopic Dermatitis: A Complex and Evolving Relationship. Trends Microbiol. 2018 Jun 1;26(6):484–97.

25. Hachem JP, Crumrine D, Fluhr J, Brown BE, Feingold KR, Elias PM. pH directly regulates

438 epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion.
439 J Invest Dermatol. 2003;121(2):345–53.

440 26. Marples RR. Sex, constancy, and skin bacteria. Arch Dermatol Res. 1982;272(3–
441 4):317–20.

442 27. Gao Z, Tseng C -h., Pei Z, Blaser MJ. Molecular analysis of human forearm superficial
443 skin bacterial biota. Proc Natl Acad Sci. 2007;104(8):2927–32.

444 28. Grice EA, Kong HH, Renaud G, Young AC, NISC Comparative Sequencing Program,
445 Bouffard GG, et al. A diversity profile of the human skin microbiota. Genome Res.
446 2008;18:1043–50.

447 29. Fierer N, Hamady M, Lauber CL, Knight R. The influence of sex, handedness, and
448 washing on the diversity of hand surface bacteria. Proc Natl Acad Sci U S A.
449 2008;105(46):17994–9.

450 30. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. Bacterial Community
451 Variation in Human Body Habitats Across Space and Time. Science (80- ). 2009
452 Dec;326(5960):1694–7.

453 31. Oh J, Byrd AL, Deming C, Conlan S, Kong HH, Segre JA, et al. Biogeography and
454 individuality shape function in the human skin metagenome. Nature.
455 2014;514(7520):59–64.

456 32. Tomida S, Nguyen L, Chiu BH, Liu J, Sodergren E, Weinstock GM, et al. Pan-genome
457 and comparative genome analyses of Propionibacterium acnes reveal its genomic
458 diversity in the healthy and diseased human skin microbiome. MBio. 2013;4(3).

459 33. Bowen AC, Tong SYC, Chatfield MD, Carapetis JR. The microbiology of impetigo in
460 indigenous children: associations between Streptococcus pyogenes, Staphylococcus

461 aureus, scabies, and nasal carriage. BMC Infect Dis. 2014 Dec 31;14(1):727.

462 34. Salah LA, Faergemann J. A retrospective analysis of skin bacterial colonisation,
463 susceptibility and resistance in atopic dermatitis and impetigo patients. Acta Derm
464 Venereol. 2015;95(5):532–5.

465 35. Matard B, Donay JL, Resche-Rigon M, Tristan A, Farhi D, Rousseau C, et al. Folliculitis
466 decalvans is characterized by a persistent, abnormal subepidermal microbiota. Exp
467 Dermatol. 2020 Mar 15;29(3):295–8.

468 36. Benzecry V, Grancini A, Guanziroli E, Nazzaro G, Barbareschi M, Marzano A V., et al.
469 Hidradenitis suppurativa/acne inversa: a prospective bacteriological study and review
470 of the literature. G Ital di Dermatologia e Venereol. 2020 Oct 1;155(4):459–63.

471 37. Tett A, Pasolli E, Farina S, Truong DT, Asnicar F, Zolfo M, et al. Unexplored diversity
472 and strain-level structure of the skin microbiome associated with psoriasis. npj
473 Biofilms Microbiomes. 2017;3(1):14.

474 38. Nowicka D, Grywalska E. Staphylococcus aureus and Host Immunity in Recurrent
475 Furunculosis. Vol. 235, Dermatology. S. Karger AG; 2019. p. 295–305.

476 39. Dréno B. What is new in the pathophysiology of acne, an overview. Journal of the
477 European Academy of Dermatology and Venereology. 2017.

478 40. Fitz-Gibbon S, Tomida S, Chiu BH, Nguyen L, Du C, Liu M, et al. Propionibacterium
479 acnes strain populations in the human skin microbiome associated with acne. J Invest
480 Dermatol. 2013 Sep 1;133(9):2152–60.

481 41. Barnard E, Shi B, Kang D, Craft N, Li H. The balance of metagenomic elements shapes
482 the skin microbiome in acne and health. Sci Rep. 2016 Dec 21;6(1):1–12.

42. Polavarapu N, Ogilvie MP, Panthaki ZJ. Microbiology of burn wound infections. J Craniofac Surg. 2008 Jul;19(4):899–902.

43. Stevens DL, Bryant AE. Impetigo, Erysipelas and Cellulitis. Streptococcus pyogenes: Basic Biology to Clinical Manifestations. University of Oklahoma Health Sciences Center; 2016.

44. Kashem SW, Kaplan DH. Skin Immunity to Candida albicans. Trends Immunol. 2016;37(7):440–50.

45. SAVOLAINEN J, LAMMINTAUSTA K, KALIMO K, VIANDER M. Candida albicans and atopic dermatitis. Clin Exp Allergy. 1993 Apr 1;23(4):332–9.

46. Wanke I, Skabytska Y, Kraft B, Peschel A, Biedermann T, Schittek B. Staphylococcus aureus skin colonization is promoted by barrier disruption and leads to local inflammation.

47. De Benedetto A, Rafaels NM, McGirt LY, Ivanov AI, Georas SN, Cheadle C, et al. Tight junction defects in patients with atopic dermatitis. J Allergy Clin Immunol. 2011 Mar 15;127(3):773-786.e7.

48. Sano S. Psoriasis as a barrier disease. Vol. 33, Dermatologica Sinica. Elsevier Ltd; 2015. p. 64–9.

49. Kong HH, Oh J, Deming C, Conlan S, Grice EA, Beatson MA, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome Res. 2012 May 6;22(5):850–9.

50. Assarsson M, Söderman J, Dienus O, Seifert O. Significant Differences in the Bacterial Microbiome of the Pharynx and Skin in Patients with Psoriasis Compared with Healthy Controls. Acta Derm Venereol. 2020 Sep 1;100(16):adv00273.

506 51. Fyhrquist N, Muirhead G, Prast-Nielsen S, Jeanmougin M, Olah P, Skoog T, et al.
507 Microbe-host interplay in atopic dermatitis and psoriasis. Nat Commun. 2019 Oct
508 16;10(1):4703.

509 52. Spacova I, Ceuppens JL, Seys SF, Petrova MI, Lebeer S. Probiotics against airway
510 allergy: host factors to consider. Dis Model Mech. 2018;11(7).

511 53. Haahtela T. A biodiversity hypothesis. Allergy. 2019 Apr 4;74(8):all.13763.

512 54. Douwes J, Cheng S, Travier N, Cohet C, Niesink A, McKenzie J, et al. Farm exposure in
513 utero may protect against asthma, hay fever and eczema. Eur Respir J.
514 2008;32(3):603–11.

515 55. Roslund MI, Puhakka R, Grönroos M, Nurminen N, Oikarinen S, Gazali AM, et al.
516 Biodiversity intervention enhances immune regulation and health-associated
517 commensal microbiota among daycare children. Sci Adv. 2020;6(42):1–11.

518 56. Nutten S. Atopic dermatitis: Global epidemiology and risk factors. Ann Nutr Metab.
519 2015;66(1):8–16.

520 57. FEDRA - Research project [Internet]. Biodiversity at School Environments Benefits for
521 ALL (B@SEBALL). [cited 2020 Nov 29]. Available from:
522 http://www.belspo.be/belspo/fedra/proj.asp?l=eb&COD=B2%2F191%2FP3%2F+B%40
523 SEBALL

524 58. Totté JEE, van der Feltz WT, Hennekam M, van Belkum A, van Zuuren EJ, Pasmans
525 SGMA. Prevalence and odds of S *taphylococcus aureus* carriage in atopic dermatitis: a
526 systematic review and meta-analysis. Br J Dermatol. 2016 Oct 1;175(4):687–95.

527 59. Byrd AL, Deming C, Cassidy SKB, Harrison OJ, Ng WI, Conlan S, et al. Staphylococcus
528 aureus and Staphylococcus epidermidis strain diversity underlying pediatric atopic

dermatitis. Sci Transl Med. 2017 Jul 5;9(397).

60. Iwamoto K, Moriwaki M, Niitsu Y, Saino M, Takahagi S, Hisatsune J, et al. Staphylococcus aureus from atopic dermatitis skin alters cytokine production triggered by monocyte-derived Langerhans cell. J Dermatol Sci. 2017 Dec 1;88(3):271–9.

61. Moriwaki M, Iwamoto K, Niitsu Y, Matsushima A, Yanase Y, Hisatsune J, et al. *Staphylococcus aureus* from atopic dermatitis skin accumulates in the lysosomes of keratinocytes with induction of IL-1α secretion via TLR9. Allergy. 2019 Mar 1;74(3):560–71.

62. Schlievert PM, Case LC, Strandberg KL, Abrams BB, Leung DYM. Superantigen profile of Staphylococcus aureus isolates from patients with steroid-resistant atopic dermatitis. Clin Infect Dis. 2008 May 15;46(10):1562–7.

63. Christensen GJM, Brüggemann H. Bacterial skin commensals and their role as host guardians. Vol. 5, Beneficial Microbes. Wageningen Academic Publishers; 2014. p. 201–15.

64. Higaki S. Lipase inhibitors for the treatment of acne. Vol. 22, Journal of Molecular Catalysis B: Enzymatic. 2003. p. 377–84.

65. Johnson T, Kang D, Barnard E, Li H. Strain-Level Differences in Porphyrin Production and Regulation in Propionibacterium acnes Elucidate Disease Associations. mSphere. 2016 Feb 25;1(1).

66. Brandi J, Cheri S, Manfredi M, Di Carlo C, Vita Vanella V, Federici F, et al. Exploring the wound healing, anti-inflammatory, anti-pathogenic and proteomic effects of lactic acid bacteria on keratinocytes. Sci Rep. 2020 Dec 1;10(1):11572.

552 67. Mohammedsaeed W, McBain AJ, Cruickshank SM, O'Neill CA. Lactobacillus rhamnosus
553 GG inhibits the toxic effects of Staphylococcus aureus on epidermal keratinocytes.
554 Appl Environ Microbiol. 2014 Sep;80(18):5773–81.

555 68. Mohammedsaeed W, Cruickshank S, McBain AJ, O'Neill CA. Lactobacillus rhamnosus
556 GG Lysate Increases Re-Epithelialization of Keratinocyte Scratch Assays by Promoting
557 Migration. Sci Rep. 2015 Dec 5;5(1):16147.

558 69. Blanchet-Réthoré S, Bourdès V, Mercenier A, Haddar CH, Verhoeven PO, Andres P.
559 Effect of a lotion containing the heat-treated probiotic strain Lactobacillus johnsonii
560 NCC 533 on Staphylococcus aureus colonization in atopic dermatitis. Clin Cosmet
561 Investig Dermatol. 2017 Jul 3;10:249–57.

562 70. Nakatsuji T, Chiang HI, Jiang SB, Nagarajan H, Zengler K, Gallo RL. The microbiome
563 extends to subepidermal compartments of normal skin. Nat Commun. 2013;4.

564 71. Spacova I, O'Neill C, Lebeer S. Lacticaseibacillus rhamnosus GG inhibits infection of
565 human keratinocytes by Staphylococcus aureus through mechanisms involving cell
566 surface molecules and pH reduction . Benef Microbes. 2020 Oct 21;1–14.

567 72. Khmaladze I, Butler É, Fabre S, Gillbro JM. *Lactobacillus reuteri* <scp>DSM</scp>
568 17938—A comparative study on the effect of probiotics and lysates on human skin.
569 Exp Dermatol. 2019 Jul 15;28(7):822–8.

570 73. Tang SC, Yang JH. Dual effects of alpha-hydroxy acids on the skin. Vol. 23, Molecules.
571 MDPI AG; 2018.

572 74. Matsubara VH, Wang Y, Bandara HMHN, Mayer MPA, Samaranayake LP. Probiotic
573 lactobacilli inhibit early stages of Candida albicans biofilm development by reducing
574 their growth, cell adhesion, and filamentation. Appl Microbiol Biotechnol. 2016 Jul

575 1;100(14):6415–26.

576 75. Allonsius CN, van den Broek MFL, De Boeck I, Kiekens S, Oerlemans EFM, Kiekens F, et
577 al. Interplay between Lactobacillus rhamnosus GG and Candida and the involvement
578 of exopolysaccharides. Microb Biotechnol. 2017 Nov 1;10(6):1753–63.

579 76. Allonsius CN, Vandenheuvel D, Oerlemans EFM, Petrova MI, Donders GGG, Cos P, et
580 al. Inhibition of Candida albicans morphogenesis by chitinase from Lactobacillus
581 rhamnosus GG. Sci Rep. 2019;9(1):1–12.

582 77. Bowe WP, Filip JC, DiRienzo JM, Volgina A, Margolis DJ. Inhibition of
583 propionibacterium acnes by bacteriocin-like inhibitory substances (BLIS) produced by
584 Streptococcus salivarius. J Drugs Dermatol. 2006;5(9):868–70.

585 78. Oh S, Kim SH, Ko Y, Sim JH, Kim KS, Lee SH, et al. Effect of bacteriocin produced by
586 Lactococcus sp. HY 449 on skin-inflammatory bacteria. Food Chem Toxicol. 2006
587 Aug;44(8):1184–90.

588 79. Lebeer S, Vanderleyden J, De Keersmaecker SCJ. Host interactions of probiotic
589 bacterial surface molecules: Comparison with commensals and pathogens. Vol. 8,
590 Nature Reviews Microbiology. Nat Rev Microbiol; 2010. p. 171–84.

591 80. Yan F, Cao H, Cover TL, Washington MK, Shi Y, Liu LS, et al. Colon-specific delivery of a
592 probiotic-derived soluble protein ameliorates intestinal inflammation in mice through
593 an EGFR-dependent mechanism. J Clin Invest. 2011 Jun 1;121(6):2242–53.

594 81. Kim W, Lee EJ, Bae IH, Myoung K, Kim ST, Park PJ, et al. Lactobacillus plantarum-
595 derived extracellular vesicles induce anti-inflammatory M2 macrophage polarization
596 in vitro. J Extracell Vesicles. 2020 Jan 1;9(1).

597 82. Lebeer S, Oerlemans E, Claes I, Wuyts S, Henkens T, Spacova I, et al. Topical cream

598 with live lactobacilli modulates the skin microbiome and reduce acne symptoms.
599 2018;1–9.

600 83. Laborel-Préneron E, Bianchi P, Boralevi F, Lehours P, Fraysse F, Morice-Picard F, et al.
601 Effects of the Staphylococcus aureus and Staphylococcus epidermidis Secretomes
602 Isolated from the Skin Microbiota of Atopic Children on CD4+ T Cell Activation. Allen
603 IC, editor. PLoS One. 2015 Oct 28;10(10):e0141067.

604 84. De Benedetto A, Rafaels NM, McGirt LY, Ivanov AI, Georas SN, Cheadle C, et al. Tight
605 junction defects in patients with atopic dermatitis. J Allergy Clin Immunol. 2011 Dec
606 15;127(3):773-786.e7.

607 85. Sultana R, McBain AJ, O'Neill CA. Strain-dependent augmentation of tight-junction
608 barrier function in human primary epidermal keratinocytes by Lactobacillus and
609 Bifidobacterium lysates. Appl Environ Microbiol. 2013 Aug;79(16):4887–94.

610 86. Jung YO, Jeong H, Cho Y, Lee EO, Jang HW, Kim J, et al. Lysates of a probiotic,
611 lactobacillus rhamnosus, can improve skin barrier function in a reconstructed human
612 epidermis model. Int J Mol Sci. 2019 Sep 1;20(17).

613 87. Pastore S, Mascia F, Mariani V, Girolomoni G. The epidermal growth factor receptor
614 system in skin repair and inflammation. Vol. 128, Journal of Investigative
615 Dermatology. Nature Publishing Group; 2008. p. 1365–74.

616 88. Peral MC, Huaman Martinez MA, Valdez JC. Bacteriotherapy with *Lactobacillus*
617 *plantarum* in burns. Int Wound J. 2009 Feb 1;6(1):73–81.

618 89. Peral MC, Rachid MM, Gobbato NM, Huaman Martinez MA, Valdez JC. Interleukin-8
619 production by polymorphonuclear leukocytes from patients with chronic infected leg
620 ulcers treated with Lactobacillus plantarum. Clin Microbiol Infect. 2010;16(3):281–6.

90. Myles IA, Earland NJ, Anderson ED, Moore IN, Kieh MD, Williams KW, et al. First-in-human topical microbiome transplantation with Roseomonas mucosa for atopic dermatitis. JCI insight. 2018 May 3;3(9).

91. Gueniche A, Knaudt B, Schuck E, Volz T, Bastien P, Martin R, et al. Effects of nonpathogenic gram-negative bacterium *Vitreoscilla filiformis* lysate on atopic dermatitis: a prospective, randomized, double-blind, placebo-controlled clinical study. Br J Dermatol. 2008 Dec 1;159(6):1357–63.

92. Nakatsuji T, Chen TH, Narala S, Chun KA, Two AM, Yun T, et al. Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis. Sci Transl Med. 2017;9(378).