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"Advancing Eczema Care: A Microbiome-Centered Approach to Product Testing"

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1. Introduction

Eczema, and in particular atopic dermatitis (AD), is one of the most prevalent chronic inflammatory skin conditions globally. Affecting both infants and adults, eczema presents a major health burden due to its impact on quality of life and the high recurrence of symptoms. The pathogenesis of AD involves several key mechanisms: (i) Skin Barrier Dysfunction: A fundamental feature of AD is the impairment of the epidermal barrier, primarily due to mutations in the filaggrin gene. These mutations lead to increased transepidermal water loss and reduced skin hydration, facilitating the penetration of allergens and microbes [1]. (ii) Immune Dysregulation: AD is characterized by a Th2-dominant immune response, with elevated levels of cytokines such as IL-4, IL-5, and IL-13. These cytokines promote IgE production and eosinophil recruitment, contributing to inflammation and tissue damage [2]. (iii) Pruritus and Neuroimmune Interactions: Itch is a hallmark symptom of AD, often exacerbated by epidermal hyperinnervation and the release of pruritogens like IL-31. This leads to a vicious cycle of scratching and further barrier disruption [3]. (iv) Microbial Dysbiosis: Patients with AD frequently exhibit an altered skin microbiome, with increased colonization by *Staphylococcus aureus* (*S. aureus*) during flare-ups [4,5]. Studies indicate that up to 70% of skin lesions in patients with AD are colonized by *S. aureus*, with nasal mucosa also frequently involved. This high colonization rate correlates with disease severity and exacerbates inflammatory processes [6]. Additionally, meta-analysis found that the pooled prevalence of *S. aureus* carriage among AD patients is 70% for lesional skin, 39% for nonlesional skin, and 62% for the nose. Notably, the prevalence of colonization in lesional skin increases with disease severity. Furthermore, a study on Korean AD patients reported that acute skin lesions had a higher colonization rate (74%) compared to chronic lesions (38%). The density of *S. aureus* was also found to be correlated with the degree of cutaneous inflammation and the severity of the AD lesion [7]. These findings underscore the significant role of *S. aureus* in the pathogenesis of AD, highlighting the importance of addressing bacterial colonization in the management of the condition.

The interaction between cosmetic or dermatological products and the skin microbiome is a critical yet under-evaluated factor in product development. Given that these products can potentially exacerbate or alleviate symptoms by influencing microbiome composition, there is an urgent need for tools that can assess their microbiome impact. In this context, the primary objective of our research was to establish a scientifically rigorous and standardized method for evaluating the microbiome-friendliness of cosmetic products formulated for eczematic skin.

This paper introduces the MyMicrobiome Eczema Standard—a new evaluation framework specifically designed to assess the compatibility of products with eczematic skin microbiota. We apply this standard to evaluate a newly developed leave-on treatment formulated for eczema, presenting both the testing methodology and outcome-based insights.

2. Materials and Methods

The MyMicrobiome Eczema Standard is a specialized framework assessing the impact of cosmetic products on the skin microbiome, with a focus on strains linked to eczema. Central to the standard is the evaluation of nine *Staphylococcus aureus* strains, often associated with atopic dermatitis, alongside beneficial commensals that maintain skin balance.

A key element is the microbial balance test, assessing the interaction between *S. aureus* and the protective commensal *Staphylococcus hominis*, which produces antimicrobial peptides and helps regulate pH. This relationship is critical, as other commensals like *S. epidermidis* may sometimes worsen inflammation in eczema [8].

The standard includes the following test areas:

- Microbiological Quality Control: Products must be free from contamination.
- Impact on Microbial Balance: The product should reduce *S. aureus* while supporting *S. hominis*.
- Preservation of Microbial Diversity: Products must maintain native microbial diversity.
- Growth Dynamics of Key Microbes: Assesses effect on the growth of beneficial species.
- Selective Inhibition of *S. aureus*: Overall the growth of nine strains must be reduced growth without harming commensals

The MyMicrobiome Eczema Standard includes two distinct test protocols—Eczema Infant 27.11 and Eczema Adult 27.10—which are specifically designed to reflect the unique microbial profiles associated with different age groups and skin regions. Each standard incorporates tailored microbial panels and testing procedures to ensure relevance and accuracy in evaluating product effects.

Eczema Infant 27.11

This protocol evaluates product impact on the infant skin microbiome through:

- Balance Assay: Measures *S. hominis* vs. *S. aureus* interaction.
- Diversity & Contact Assays (Direct & Indirect): Simulate exposure using: *L. crispatus, S. epidermidis, S. mitis, C. tuberculostearicum*, and *C. acnes*
- Vitality Assay for *S. aureus*: Assesses product efficacy against 9 strains: DSM 346, 799, 3463, 6148, 11823, 110566, 111212, 113529, 113533

Eczema Adult 27.10

This version reflects the microbial diversity of adult skin, tailored to different skin regions:

- Balance Assay: Focuses on *S. hominis* vs. *S. aureus*.
- Diversity & Contact Assays for Sebaceous Skin:
  C.acnes, S.epidermidis, S. capitis, S. hominis, S. mitis, C. simulans, Malassezia spp.
- Diversity & Contact Assays for Moist Skin:
  C.tuberculostearicum, C. simulans, S. epidermidis, S. capitis, S. hominis, C. acnes, Malassezia spp.
- Pathogenic Vitality Assay for *S. aureus*: Evaluates the same 9 strains as mentioned above.

All strains were sourced from DSMZ (Germany) and cultured on standard media: CASO broth and TSA at 37°C; *Malassezia* was grown on lipid-specific agar at 30°C.

2.2 Product Sample Preparation and Controls

The test product was an eczema treatment product formulated for leave-on application (no dilution before use). It has the following ingredients: Aqua, Phage Lysate, Hydroxyethylcellulose, Niacinamide.

For assays requiring mixing with microbial suspensions (liquid culture assays described below), a defined amount of product was dispersed in PBS and/or broth to create a uniform test suspension. Specifically, in the suspension-based assays, the product was thoroughly mixed with sterile PBS and nutrient broth as indicated for each assay (e.g., CASO broth) to simulate the product's interaction with microbes in a fluid environment. In surface contact assays, the product was applied directly onto the agar surface. In all assays, parallel control samples were prepared in which the product was replaced by an equal volume of PBS. These PBS-only controls were included to account for natural microbial growth or death in the absence of product and to provide a baseline for comparison. All materials (tubes, pipette tips, agar plates) were sterile, and all preparations were performed aseptically to ensure reproducibility.

2.3 Microbiological Quality Test of the Product

Before assessing the product's impact on the microbiome, its microbiological quality was verified according to ISO 17516:2015 to ensure it was contamination-free. A 100 µL sample was plated on non-selective media for total aerobic microbial count (TAMC) and yeast and mold count (TYMC). Plates were incubated under standard conditions (32–37°C for 4 days for bacteria, 25–30 °C for up to 7 days for fungi). If no microbial growth is detected, the absence of contaminants is confirmed, which ensures that subsequent effects were due solely to intentional inoculation.

2.4 *S. hominis* – *S. aureus* Balance Co-culture Assay

Bacterial suspensions were standardized to have the desired CFU/mL. For each assay, the test product was mixed with medium and the bacterial suspension. A negative control replaced the product with PBS under identical conditions. Samples were incubated at 32 ± 2°C with agitation for 4 hours (leave-on simulation). Aliquots were taken at 0 minutes and 4 hours, then plated in triplicate on appropriate agar. Colonies were identified by morphology and growth, and CFU/mL was calculated to determine bacterial reduction relative to the control.

2.5 Mixed Microbial Community Diversity Assay

Bacterial suspensions were prepared by adjusting each strain to the appropriate colony-forming units per milliliter (CFU/mL), depending on the species' physiological characteristics. Equal volumes of the individual microbial suspensions were then combined to create a standardized mixed microbial inoculum.

For each test condition, the product was added to a distinct medium to form the test batch. Subsequently, the mixed microbial suspension was inoculated into the product-medium mixture. A control batch was prepared in parallel, in which sterile phosphate-buffered saline (PBS) was used in place of the product, while maintaining identical preparation conditions.

All samples were incubated at 32°C ± 2°C under continuous agitation for 4 hours (simulating leave-on application). At each designated time point—t = 0 h and t = 4 h—aliquots were taken and plated in triplicate onto appropriate culture media for quantitative microbial analysis.

The agar plates were incubated under species-specific conditions: 24–48 hours at 37°C ± 2 °C for bacterial strains, and 5–7 days at 30°C ± 2 °C for *Malassezia* spp., using the appropriate atmospheric requirements for each organism.

Microbial colonies were distinguished based on their morphological characteristics, including colony shape, color, and size, allowing for accurate visual differentiation and enumeration of each species. The colony-forming units per milliliter (CFU/mL) were calculated for each test organism, and the results were expressed as ratios between product-treated and control conditions, providing a quantitative measure of the product's impact on microbial composition and viability.

2.6 Simulation of Skin–Product Contact: Assessment of Direct and Indirect Microbial Interaction

To evaluate the impact of cosmetic formulations on skin-resident microorganisms under conditions mimicking topical application, a dual assay system was employed to model transient (direct) contact and core (indirect) contact with the skin microbiome.

2.6.1 Transient Microbiome Model (Direct Contact Assay)

To simulate immediate contact between a topical product and the transient microbiome—microorganisms residing on the skin's surface—each microbial strain was cultured and adjusted to the appropriate CFU/mL, based on species-specific growth requirements.

For each test:

- The microbial suspension was aseptically plated in triplicate onto solidified nutrient agar, forming a uniform bacterial layer.
- Following initial absorption of the bacterial suspension into the agar, test product was carefully applied directly onto the center of each inoculated plate.
- A negative control was included for each strain, in which sterile phosphate-buffered saline (PBS) was applied instead of the product.

The plates were incubated under optimal growth conditions specific to each microorganism:

- Bacterial strains: 24–72 hours at 37°C ± 2°C.
- *Malassezia* species: 5–7 days at 30°C ± 2°C in a humidified chamber with a lipid-supplemented environment to support fungal growth.

This setup replicates the short-term exposure of surface microbiota to topical applications and allows visual assessment of any inhibitory effects directly beneath and surrounding the area of product application.

2.6.2 Core Microbiome Model (Indirect Contact Assay)

To simulate interactions with the core microbiome—microorganisms embedded deeper in the skin layers or within biofilms—an overlay method was employed:

- Each microbial strain was adjusted to the appropriate CFU/mL.
- The bacterial suspension was plated onto agar in triplicate and immediately overlaid with top agar to entrap the organisms beneath a semi-solid matrix.
- After solidification of the top agar layer, the test product was gently applied to the surface.
- For each strain, a PBS-treated control was prepared in parallel by replacing the product sterile PBS.

Incubation conditions mirrored those used for the direct contact assay:

- Bacteria: 24–72 hours at 37 °C ± 2°C.
- *Malassezia* spp.: 5–7 days at 30°C ± 2°C under appropriate humidity and lipid-enriched conditions.

This assay configuration evaluates whether the product exerts inhibitory effects via diffusion or vapor-phase interactions, simulating exposure of sub-surface or biofilm-embedded microbes to topical agents. In both assay formats, the effect of the product on microbial viability was assessed by visually inspecting the plates for zones of growth inhibition, changes in colony morphology, or density reductions. This dual-assay strategy provides a comprehensive understanding of the product's compatibility with both the surface and embedded constituents of the skin microbiome.

2.7 *S. aureus* Reduction Assay

Bacterial suspensions were standardized to the appropriate CFU/mL. For each assay, the test product was mixed with medium and the bacterial suspension. A negative control replaced the product with PBS under identical conditions. Samples were incubated at 32°C ± 2°C with agitation for 4 hours (leave-on simulation). Aliquots were taken at 0 minutes and 4 hours, then plated in triplicate on appropriate agar. CFU/mL was calculated to determine bacterial reduction relative to the control. Morphological changes due to phage lysis were also monitored.

All experiments were performed *in vitro* using laboratory-grown microbial cultures. No animal testing was conducted at any stage of this study, in full compliance with IFSCC guidelines which prohibit the use of animal testing.

3. Results Adult Skin (Standard 27.10) and Infant Skin (Standard 27.11)

The product was assessed according to the MyMicrobiome Standard 27.10 for adult skin and 27.11 for Infant Skin and successfully met all evaluation criteria, earning the Microbiome-friendly certification. Below is a summary of the key findings.

3.1 Microbiological Quality

The tested product met microbiological quality criteria as defined by DIN ISO 17516, with total aerobic microbial count (TAMC) and total combined yeast/mold count (TYMC) below 20 CFU/0.1 mL, confirming the absence of microbial contamination.

3.2 Skin Microbiome Balance (*S. hominis* / *S. aureus* Co-culture)

In the microbial balance assay, the product maintained viability of the beneficial commensal *S.hominis* (1.3 × 10⁴ CFU/mL) while entirely inhibiting *S. aureus* (0 CFU/mL) after 4 h of incubation (Fig. 2). The resulting product/control ratio was 0.0 for *S. aureus*, corresponding to a rating of 1, indicating strong antagonistic effect without disruption of commensal viability (Fig. 1).

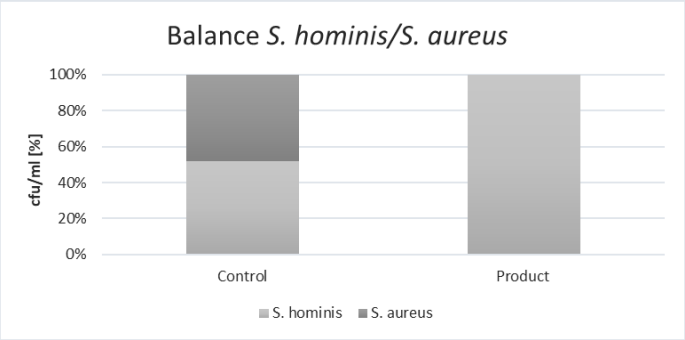

Figure 1 Balance *S.hominis*/*S.aureus*

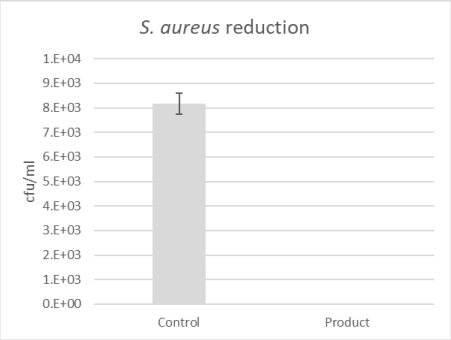

Figure 2 *S.aureus* reduction

3.3 Microbial Diversity

The product's effect on microbial diversity was assessed using a representative infant skin and adult (moist and sebaceous skin sites) microbiota. A co-culture of key organisms of the specific body region is incubated with the product for 4 h. CFU were counted, and the ratio of the CFU in the presence of the product compared to the control (PBS) were determined.

The overall diversity rating was 2.6 for infant skin, reflecting mixed outcomes across species. While three species were increased in growth, two were signigficantly reduced (*S. mitis* and *C. acnes*).

The product preserved most of the typical skin microbiota in both sebaceous and moist skin environments of adult skin. In the sebaceous skin model, some reduction in *S. capitis* and *S. epidermidis* was noted, resulting in a diversity score of 1.9. In the moist skin model, most microbes remained stable, with *C. simulans* showing an increase while *Malassezia spp.* showing slight reduction. This also resulted in a score of 1.9, indicating acceptable microbiome diversity retention.

3.4 Growth Behavior of Commensals – Direct and Indirect Contact

The influence of the product on the growth of each individual key organism of the specific body region is investigated and the ratio of the CFU in the presence of the product is calculated in % relative to the control sample (PBS). Product contact with microorganisms is direct.

Direct Contact Assay on the Infant Skin Panel yielded an overall rating of 1.6. Notably, *L. crispatus*, *S. mitis*, and *C. tuberculostearicum* showed stable growth, while *C. acnes* exhibited a reduced colony count.

Indirect Contact Assay showed minimal interference with microbial viability (overall rating: 1.2), indicating excellent compatibility with commensal organisms under non-direct exposure.

For the species of adult skin under direct contact conditions, minor reductions in *C. acnes*, *S. epidermidis*, and *C. simulans* were observed, yielding an overall score of 2.0 for both sebaceous and moist skin commensals. Under indirect contact conditions, commensal growth was largely stable, with a score of 1.3 in both skin models. This suggests that the product has a gentle effect on beneficial skin flora, especially when not applied directly.

3.5 Inhibition of *S. aureus* Strains

The antimicrobial performance of the product was evaluated against nine clinically relevant *S. aureus* strains. The product demonstrated strong inhibition across the majority of tested strains (Fig. 3, Tab. 1). This comprehensive reduction indicates a strong antimicrobial effect, shown through reduction and lysis of colonies. The overall rating for this assay was 2.0, meeting the requirement for microbiome-friendliness in this category.

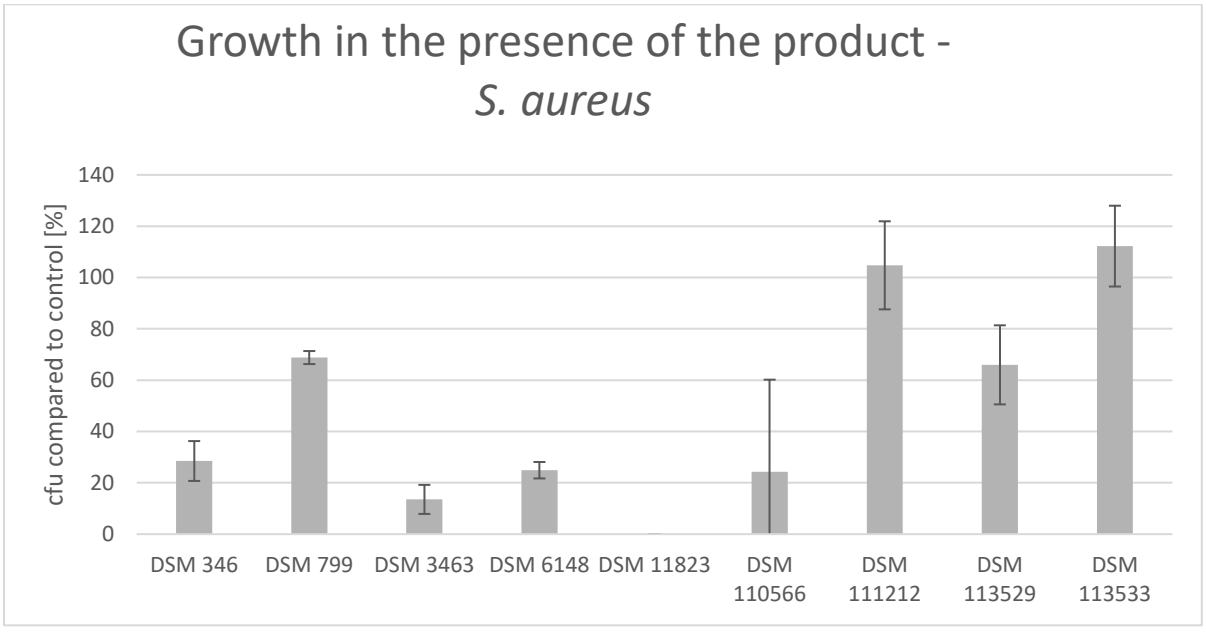

Figure 3: Growth reduction of 9 *S. aureus* strains

Table 1: Growth reduction of 9 *S. aureus* strains

Key-Microbe	cfu /Plate		Lysis	Rating
DSM 346	Control	207.0		
	Product	59.0	yes	1
DSM 799	Control	121.3		
	Product	83.5	yes	3
DSM 3463	Control	92.3		
	Product	12.5	yes	1
DSM 6148	Control	191.3		
	Product	47.7	yes	1
DSM 11823	Control	257.7		
	Product	0.0	yes	1
DSM 110566	Control	121.7		
	Product	29.5	no	2
DSM 111212	Control	28.0		
	Product	29.3	yes	3
DSM 113529	Control	193.0		
	Product	127.3	yes	3
DSM 113533	Control	16.3		
	Product	18.3	no	3
Overall rating:			2.0	

3.6 Certification Outcome

The results are evaluated using a grading scale from 1 (highest performance) to 3 (lowest performance). To pass, a product must achieve grades between 1.0 and 2.0 in the categories of *S. aureus* reduction in Balance and Contact, as well as demonstrate favorable outcomes in Balance (product/control ratio), Diversity, and Direct and Indirect Contact. The tested product successfully met all criteria, achieving required scores for *S. aureus* reduction and satisfying the overall microbiome-friendliness requirements.

4. Discussion

The skin microbiome plays a key role in the development and severity of atopic dermatitis (AD). An imbalance—characterized by reduced microbial diversity and increased *Staphylococcus aureus* colonization—is consistently linked to flares and chronic inflammation [9, 10]. Our findings support this and were clinically validated: the tested product inhibited *S. aureus* while preserving *Staphylococcus hominis*, a beneficial skin bacterium known to produce antimicrobial peptides and suppress *S. aureus* virulence [11] and relieving eczema symptoms in patients (data not shown).

The MyMicrobiome Eczema Standard approaches product evaluation from an ecological perspective, focusing on the skin as a living ecosystem. It assesses how formulations influence not only the presence of *S. aureus* but also the viability of beneficial microbes and the overall diversity of the skin microbiota under realistic skin-like conditions. In both infant and adult skin models, the product tested preserved microbial diversity—an essential indicator of skin health, as diversity loss is associated with worsening AD symptoms [12].

These findings support the MyMicrobiome Eczema Standard as a meaningful and ecologically informed tool for evaluating skin care compatibility for eczematic skin. The products efficacy on eczematic skin was confirmed in clinical trials (data not shown).

5. Conclusion

This study reinforces the critical role of the skin microbiome in the context of atopic dermatitis and highlights the value of a microbiome-focused evaluation standard. The test results demonstrate that the evaluated formulation successfully reduced *S. aureus*—a known driver of eczema flares—while maintaining the viability of beneficial strains such as *S. hominis*. Furthermore, the formulation showed minimal impact on microbial diversity across both infant and adult skin models. The *in vitro* data were supported by clinical outcomes with the same product.

The application of the MyMicrobiome Eczema Standard offers a new and comprehensive approach to assessing product compatibility with eczematic skin. By treating the skin as a living ecosystem and evaluating the interplay between harmful and beneficial microbes, the standard goes beyond traditional safety assessments. This aligns with the increasing recognition that maintaining microbial balance is essential to supporting skin barrier function, regulating immune responses, and ultimately reducing disease burden in individuals with AD.

These findings provide a solid foundation for the adoption of microbiome-based certification as a meaningful benchmark for product development and consumer trust. Going forward, further research is encouraged to assess the long-term effects of microbiome-friendly products on clinical outcomes and to expand testing approaches to reflect the full complexity of the human skin microbiome.

6. References

1. Boothe, W. D., Tarbox, J.A., Tarbox, M. B. (2024). *Atopic Dermatitis: Pathophysiology*. Adv Exp Med Biol. 1447:21-35. https://doi.org/10.1007/978-3-031-54513-9_3

2. Yue, C., Zhou, H., Wang, X., Yu, J., Hu, Y., Zhou, P., Zhao, F., Zeng, F., Li, G., Li, Y., Feng, Y., Sun, X., Huang, S., He, M., Wu, W., Huang, N., Li, J. 2020) *Atopic dermatitis: pathogenesis and therapeutic intervention.* MedComm. 8;5(12):e70029. https://doi.org/10.1002/mco2.70029.

3. Rerknimitr, P., Otsuka, A., Nakashima, C. *et al.* (2017). *The etiopathogenesis of atopic dermatitis: barrier disruption, immunological derangement, and pruritus.* Inflamm Regener. 37, 14. https://doi.org/10.1186/s41232-017-0044-7

4. Byrd, A. L., Belkaid, Y., & Segre, J. A. (2018). *The human skin microbiome*. Nature Reviews Microbiology, *16*, 143–155. https://doi.org/10.1038/nrmicro.2017.157 Sanford, J. A., & Gallo, R. L. (2013). *Functions of the skin microbiota in health and disease*. Seminars in Immunology, *25*(5), 370–377. https://doi.org/10.1016/j.smim.2013.09.005

5. Totté, J.E.E., Van der Feltz, W.T., Hennekam, M., Van Belkum, A., Van Zuuren, E.J. S.G.M.A. Pasmans (2016). *Prevalence and odds of* Staphylococcus aureus *carriage in atopic dermatitis: a systematic review and meta-analysis.* British Journal of Dermatology. 175, 4, 663-838, e122-e131. https://doi.org/10.1111/bjd.14566

6. Park, H. Y., Kim, C. R., Huh, I. S., Jung, M. Y., Seo, E. Y., Park, J. H., Lee, D. Y., Yang, J. M. (2013) Staphylococcus aureus *colonization in acute and chronic skin lesions of patients with atopic dermatitis*. Ann Dermatol. 25(4):410-416. https://doi.org/ 10.5021/ad.2013.25.4.410.

7. Kong, H. H., Oh, J., Deming, C., Conlan, S., Grice, E. A., Beatson, M. A., ... & Segre, J. A. (2012). *Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis*. Genome Research, *22*(5), 850–859. https://doi.org/10.1101/gr.131029.111

8. Cau, L., Williams, M., Butcher, A., Nakatsuji, T., Kavanaugh, J., Cheng, J., Shafiq, F., Higbee, K., Hata, T., Horswill, A., & Gallo, R. (2020). Staphylococcus epidermidis *protease EcpA can be a deleterious component of the skin microbiome in atopic dermatitis. The Journal of allergy and clinical immunology*. https://doi.org/10.1016/j.jaci.2020.06.024.

9. Salava, A., & Lauerma, A. (2014). Role of the skin microbiome in atopic dermatitis. Clinical and Translational Allergy, 4, 33. https://doi.org/10.1186/2045-7022-4-33

10. Paller, A. S., Kong, H. H., Seed, P., Naik, S., Scharschmidt, T. C., Gallo, R. L., Luger, T. A., & Irvine, A. D. (2018). *The microbiome in patients with atopic dermatitis.* Journal of Allergy and Clinical Immunology, 143(1), 26–35. https://doi.org/10.1016/j.jaci.2018.11.015

11. Severn, M. M., Williams, M. R., Shahbandi, A., Bunch, Z. L., Lyon, L. M., Nguyen, A., ... & Horswill, A. R. (2022). *The ubiquitous human skin commensal* Staphylococcus hominis *protects against opportunistic pathogens*. mBio, 13(3), e00930-22. https://doi.org/10.1128/mbio.00930-22

12. Wang, Z., Hülpüsch, C., Traidl-Hoffmann, C., Reiger, M., & Schloter, M. (2024). *Understanding the role of* Staphylococcus aureus *in atopic dermatitis: strain diversity, microevolution, and prophage influences.* Frontiers in Medicine, 11, 1480257. https://www.frontiersin.org/journals/medicine/articles/10.3389/fmed.2024.1480257/full