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"Effect of immediate and chronic use of biosurfactant cleanser on reactive skin and its impact on microbiome"

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1. Introduction

Facial cleansing is key in any skin care routine, helping to remove pollution, excess of sebum or make up products and dead skin cells, leaving skin fresh and clean. Dermatologists often recommend to use hygiene products that do not disrupt the skin barrier, both the hydro-lipidic layer (respect of natural hydration, sebum level and quality), the pH, the organization of intercellular lipids of the skin, the cohesion of the stratum corneum, in order to ensure a stable environment for the commensal microbiome.

However, the selection of an appropriate cleanser for sensitive skin presents a unique challenge. Sensitive skin is an important dermatologic condition, however, defining exactly what constitutes sensitive skin can be challenging. Three dermatologic conditions are commonly included in sensitive skin panels and include atopic dermatitis, rosacea, and cosmetic intolerance syndrome. Atopic dermatitis is characterized by immune hypersensitivity accompanied by a defective barrier. Rosacea is characterized by vascular hyperreactivity manifested by easy flushing/blushing, erythema, and acne of the face. Finally, cosmetic intolerance syndrome is characterized by sensory hypersensitivity manifesting as stinging, burning, itching, and tingling induced by skin care products. Thus, these three diseases address the defective barrier, vascular hyperreactivity, and sensory issues that combine to define sensitive skin. This research examined the value of a specially formulated cleanser in this challenging sensitive skin population.

In terms of formulation chassis, surfactants are the key ingredients as they are the primary cleansing agents. Chemical surfactants have been reported to have the potential to cause detrimental effects such as allergic reactions and skin irritations to the human skin; hence, there is a need for the replacement of chemical surfactants with other compounds that would have less or no negative effects on skin health.

Biosurfactants (surfactants of biological origin) have exhibited great potential such as lower toxicity, skin compatibility, protection and surface moisturizing effects which are key components for an effective skincare routine. This makes them suitable substitutes for chemical surfactants in current cosmetic and personal skincare pharmaceutical formulations. These glycolipids provide also with very gentle yet effective cleansing efficacy and foaming properties.

Glycolipids come from a bacterial fermentation process, based on the specific “pseudo- solubilization” activity of bacterial strain *pseudomonas putida.* This nature-derived biosurfactant presents an excellent ecoprofile, with 100% biodegradability and super low aquatic toxicity. This ultimate sustainable character of glycolipids aligns seamlessly with the increasing demand for environmentally conscious skincare products.

The use of living organisms as a factory gives access to its unique structure compared to other surfactants: a glycolipid with a double sugar head and a double long lipophilic chain providing both physicochemical and biological properties, such as microbiome modulation or anti-inflammation properties [6, 7, 8, 9].

Most studies linking cleansers to skin microbiome targeted healthy skin [10, 11, 12]. However it is well established that the adhesion of exogenous microorganisms to the skin surface and their colonization may cause skin infections or dysbiosis associated to skin disorders, such as atopic dermatitis [13, 14, 15, 16]. So the use of cleansers could be an opportunity to modulate the adhesion and colonization of pathogen bacteria on the skin.

Here we used the selective properties of a glycolipid biosurfactant on the bacteria *S. aureus* to formulate a cleanser dedicated to sensitive and reactive skin. We conducted a clinical study to evaluate the performance of a foaming glycolipid-based cleanser versus a non-foaming hydrating cleanser benchmark in a sensitive skin panel comprised of subjects with historical propensity to develop atopic dermatitis, rosacea, and cosmetic intolerance syndrome. We evaluated the tolerance, the clinical efficacy, the instrumental performance (hydration, barrier, redness) and the consumer perception. We evaluated the bacterial load and microbial diversity before and after a 28 days usage of these two cleansers and tried to evaluate the impact of the biosurfactant antiadhesion property on the clinical performance.

2. Materials and Methods

2.1. Test products:

In vitro studies have been performed on the biosurfactant di-rhamnolipid (INCI Glycolipid). A rhamnolipid-based autofoam cleanser, called glycolysine auto-foam cleanser, was formulated and tested against a standard non-foaming hydrating cleanser (benchmark).
Auto-foam cleanser: Glycolipid was mixed with sodium methyl cocoyl taurate and disodium cocoyl taurate, with glycerin 15%. Poly-epsilon lysine 0,2% was added as a deposition engine.
Benchmark: a non-foaming hydrating cleanser with less than 1% surfactant and a rich emollient content (7,5% glycerin, PEG-40 stearate, stearyl alcohol, cetyl alcohol, glyceryl stearate).

2.2. In vitro tests:

a) Soothing effect – PAR2 receptor-antagonist test:

The PAR2 receptor-antagonistic effect of glycolipid was assessed by measuring intracellular calcium mobilization in trypsin stimulated Hela cells. Effect of compound was compared to AZ8838, used as a reference molecule in this test. Glycolipid was tested at 8 concentrations from 0.3% (0.06 %p/p MP, 0.03 %p/p MA, ~428 µM) and dilution factor 3.

b) Anti-adhesion test versus *S aureus & S epidermidis*:

In this study, *Staphylococcus aureus* ATCC 6538 and *Staphylococcus epidermidis* ATCC 12228 reference strains were used, along with a clinical isolate of *Staphylococcus aureus* AD08cc1 obtained from an atopic dermatitis lesion [17].
Glycolipid solutions at non-bactericidal concentrations (0.5%, 1%, 1.5%, and 2% RM) or sterile distilled water (control) were applied (25 µL) to 1 cm² EpiSkin™ reconstructed human skin models and incubated for 2 hours (37°C, 5% CO_2). A 1 mL bacterial inoculum (10^7 CFU/mL in physiological water) was then applied to the epidermal surface, followed by a second 2-hour incubation under the same conditions. Non-adherent bacteria were removed by rinsing with sterile water. Adherent bacteria were recovered by sonicating (5 min, 35 kHz) the explants in 9 mL of EUGON LTSup neutralizing medium and enumerated on Tryptic Soy Agar [18, 19].

c) 3D Atopic skin model:

A reconstructed human epidermis model (SkinEthic RHE-D17) was treated with glycolipid at two subtoxic doses of 0.025 and 0.05 mg/mL. After 24h of treatment, an inflammatory Cocktail (Ckt A) composed of a combination of IL-4, IL13, TNF-α, and Poly I:C mimicking the T helper 2 (Th2) response is added for further 24h. The Ckt A treatment leads to epidermal changes such as spongiosis, inflammation and antimicrobial (increased IL-8 and S100A7 expression), and impaired barrier function (decreased filaggrin and loricrin expression). Dexamethasone, a common pharmaceutical reference, was used as a positive control.

2.3. Clinical protocol:

A clinical study was conducted on reactive skin subjects with historical propensity to develop Atopic Dermatitis (**AD)**, Rosacea (**R**), and Cosmetic Intolerance Syndrome (**CIS**). It conforms to the Declaration of Helsinki and has received appropriate ethical approvals, with informed consent form signed from each subject prior to performing any study procedure.

Eligibility:N=40 subjects for each formulation cell, 18-65 years, open ethnicity type, open Fitzpatrick (I-VI) type, Males & Females + opened to all genders.

Reactive skin panel eligibility: 100% Self-Perceived Sensitive Skin, 100% Self-Perceived Reactive Skin, Mild Cosmetic Intolerance Syndrome **(CIS)** (33% of population), Mild Atopy Dermatitis Prone (33% of population) **(AD),** Mild Rosacea (33% of population) **(R),**

Conduct of study: Dermatologist Sensitive Skin Assessment, Dermatologist Tolerance Assessment, Subject Tolerability Assessment, Dermatologist Clinical grading evaluation, Instrumental measurements (Dermalab (TEWL) ; Corneometer (Hydration) ; Spectrophotometer DSM-4 (Redness)) and Subject Assessment Questionnaires were performed at all time points (Baseline, Immediately after cleansing, 8h, 24h, 2 weeks and 4 weeks).

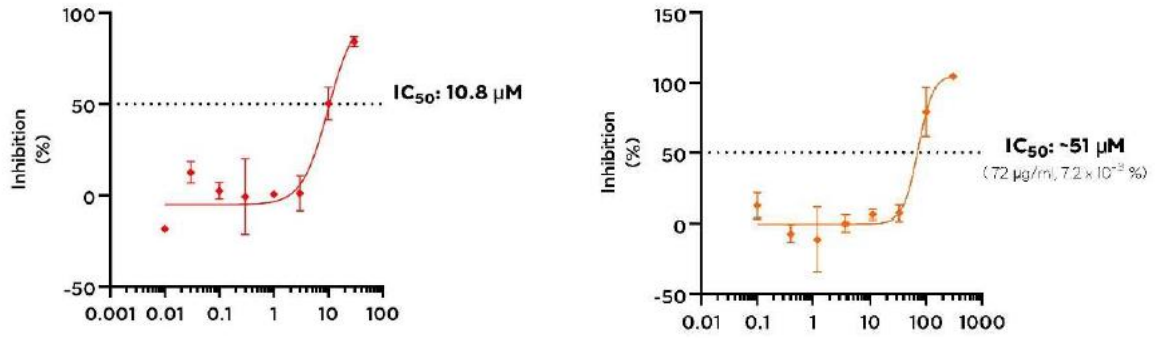
Microbiome sampling: Skin microbiome samples were collected on the cheek by swabbing at baseline, immediately, 24hours and week 4 and analyzed by quantitative PCR.

3. Results

3.1. In vitro evaluations:

a) Soothing effect – PAR2 receptor-antagonist test:

Glycolipid inhibited clearly, in a concentration dependent manner, the intracellular calcium mobilization by Trypsin stimulated Hela cells. The antagonist effect of Glycolipid (IC50 72 µg/ml, 7.2 x 10-3%, ~51 µM) was close to that of AZ8838, the pharmacological reference (Figure 1). Glycolipid is potent inhibitor of PAR2 activation.



A) [AZ8838] in µM B) [Glycolipid] in µM

Figure 1: Effect of AZ8838 (A) and Glycolipid (B) on PAR activation in Trypsin-stimulated Hela cells

b) Anti-adhesion test versus *S aureus & S epidermidis*:

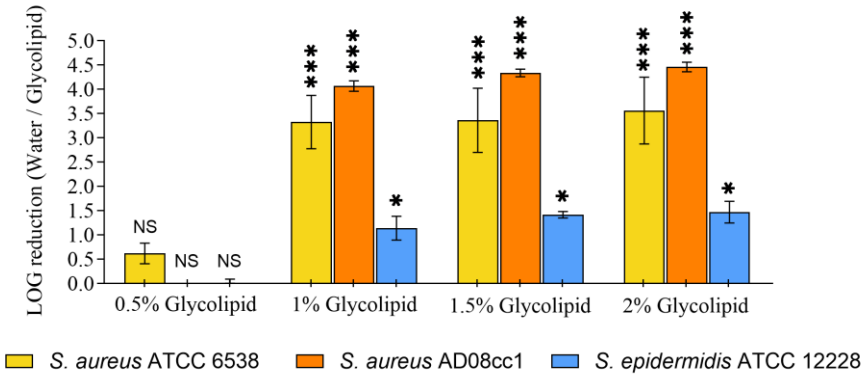


Figure 2: Impact of different glycolipid concentrations on the adhesion of skin bacteria to a 3D skin model. Results are expressed as the logarithm of reduction, *i.e*. the Log10 of the ratio of bacteria numbers adhered per cm^2 of reconstructed skin of the 3D model between the glycolipid-treated conditions and the sterile distilled water-treated condition (negative control). Error bars show standard error of the mean (SEM) of independent treatments ($n \geq 3$). Statistical significance was calculated by a paired t-test and by a multiple comparison of strains using the Tukey's test following one-way ANOVA. (NS) for non significant, (*) for $p < 0.05$, (**) for $p < 0.01$ and (***) for $p < 0.001$.

Results Antiadhesion

The anti-adhesive activity of the glycolipid was evaluated against *S. aureus* ATCC 6538, *S. aureus* $AD08_{CC1}$, and *S. epidermidis* ATCC 12228 at concentrations of 0.5%, 1%, 1.5%, and 2% in water. The glycolipid demonstrated strong anti-adhesive activity (>3 Log CFU/cm^2) against both *S. aureus* strains, while the inhibition of *S. epidermidis* adhesion was significantly weaker (<1.5 Log CFU/cm^2). This inhibition appears to be threshold-dependent, with maximal significant activity observed at concentrations of 1% and above (Figure 2).

c) 3D Atopic skin model:

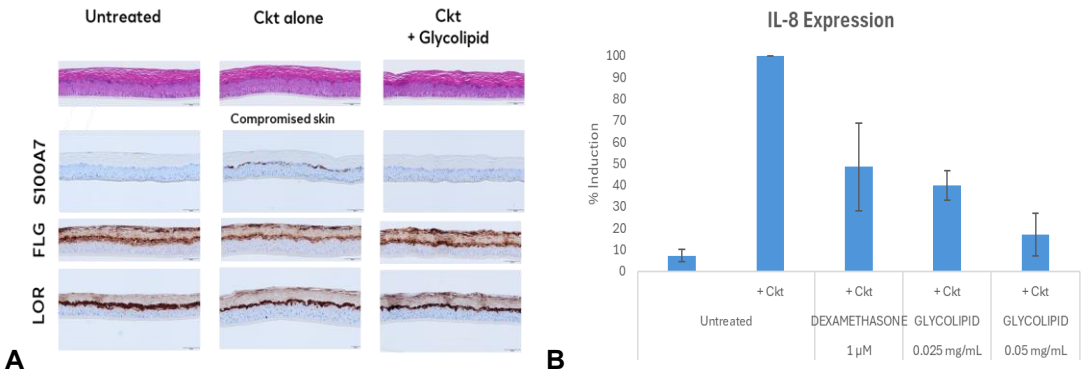
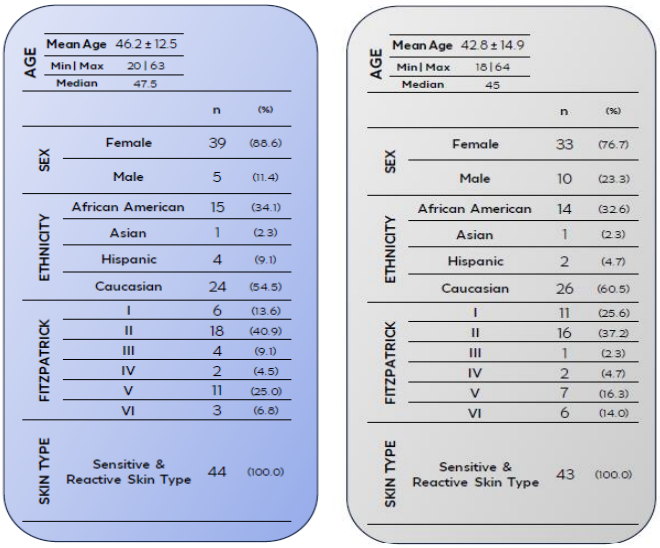


Figure 3: Morphology, Immunohistochemistry of biomarkers S100A7, Filaggrin (FLG) & Loricrin (LOR) for untreated sample, compromised skin sample with cocktail A (Ckt), treated skin sample with Glycolipid 0,05mg/ml (A) and IL-8 expression for dose effect of Glycolipid (B).

The glycolipid prevents strongly the IL-8 expression in a dose-dependent manner and the expression of S100A7 on Reconstructed Human Epidermis (Figure 3). The glycolipid at 0.05 mg/mL prevents the Ckt A effects not only on the epidermal disorganization but also on the filaggrin and loricrin expressions. Altogether these results demonstrate the benefits of the glycolipid on the inflammatory state and the barrier function of the epidermis in **AD** conditions.

3.2. Clinical study results :

a) Demographics:



		n	(%)
AGE	Mean Age 46.2 ± 12.5		
	Min | Max 20 | 63		
	Median 47.5		
SEX	Female	39	(88.6)
	Male	5	(11.4)
ETHNICITY	African American	15	(34.1)
	Asian	1	(2.3)
	Hispanic	4	(9.1)
	Caucasian	24	(54.5)
FITZPATRICK	I	6	(13.6)
	II	18	(40.9)
	III	4	(9.1)
	IV	2	(4.5)
	V	11	(25.0)
	VI	3	(6.8)
SKIN TYPE	Sensitive & Reactive Skin Type	44	(100.0)

		n	(%)
AGE	Mean Age 42.8 ± 14.9		
	Min | Max 18 | 64		
	Median 45		
SEX	Female	33	(76.7)
	Male	10	(23.3)
ETHNICITY	African American	14	(32.6)
	Asian	1	(2.3)
	Hispanic	2	(4.7)
	Caucasian	26	(60.5)
FITZPATRICK	I	11	(25.6)
	II	16	(37.2)
	III	1	(2.3)
	IV	2	(4.7)
	V	7	(16.3)
	VI	6	(14.0)
SKIN TYPE	Sensitive & Reactive Skin Type	43	(100.0)

Auto-foam Glycolysine (A) Non-foaming benchmark (B)

Figure 4: Population description for the 2 groups Glycolysine (A) and Benchmark (B) cleanser.

b) Instrumental evaluation:

Hydration: Both products demonstrated significant improvement in facial hydration at t_{imm}, 8hr & w4 across all reactive conditions (graph not shown). Glycolysine Cleanser demonstrated significantly higher facial hydration compared to Benchmark at T imm.The increased corneometry was most pronounced in atopic dermatitis (Figure 5) at Timm, 8h, 24h, and W2.

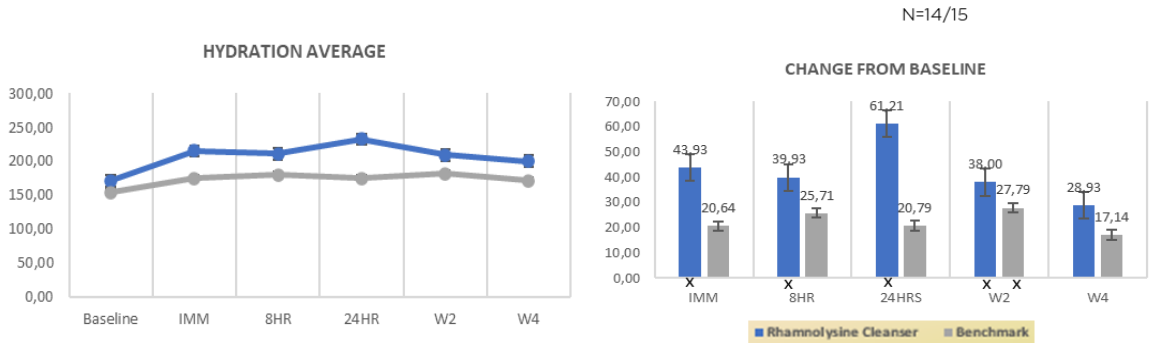


Figure 5: Corneometry for Benchmark and Glycolysine cleanser (Rhamnolysine based cleaser) in the Atopic prone skin group (X denotes statistical significance).

TEWL: Both cleansers demonstrated significant and comparable improvement in Facial TEWL at various timepoints within Cosmetic Intolerance sub-group (graph not shown).

Spectrophotometer: No significant change from baseline for both formulas for instrumental redness across all reactive conditions (graph not shown).

c) Clinical gradings and assessment:

Reactive condition clinical grading (IGA):

Both cleansers demonstrated a statistically significant reduction from baseline in Reactive Skin Conditions beginning at Week 2 across all reactive conditions. Glycolysine cleanser demonstrated statistically significant IGA reduction at Week 2 for the AD group, whereas no significant reduction for Benchmark. Both cleansers demonstrated clinically & statistically significant reduction in Reactive Skin Conditions at Week 4 for the Atopic Dermatitis Sub-Group

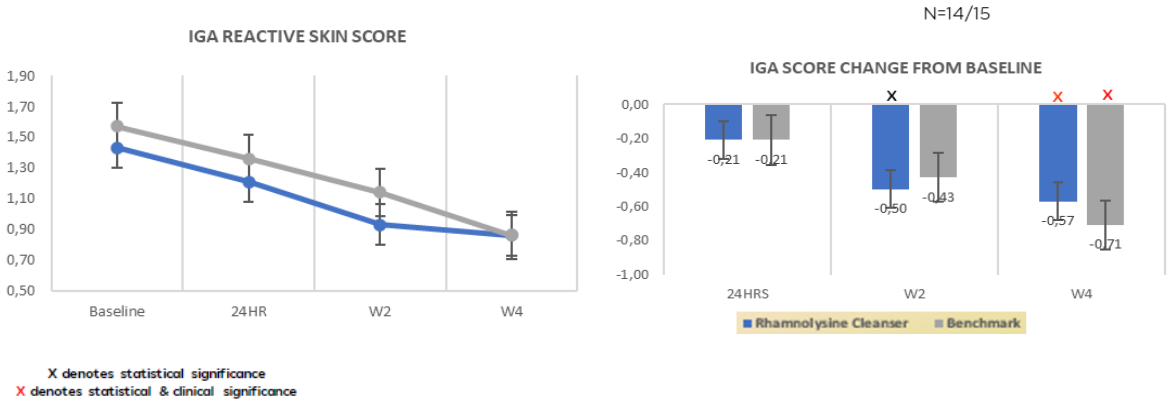


Figure 6: Clinical assessment of IGA Reactive skin score for both Benchmark and Glycolysine cleanser (rhamno-lysine based cleanser) in the Atopic prone skin group, at different time points.

Facial Dryness clinical scoring:

Glycolysine cleanser demonstrated clinical and statistically significant improvement in facial dryness at 24hours through week 4 in the Atopic Dermatitis prone group.

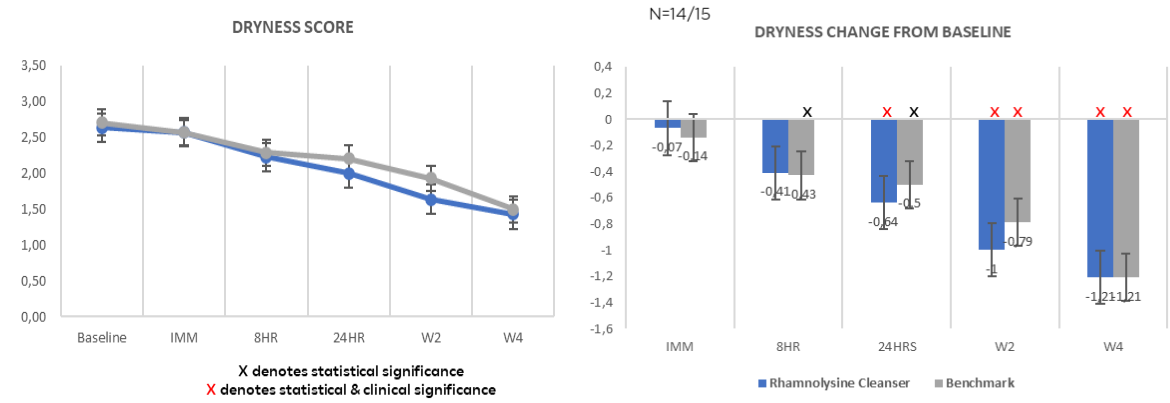


Figure 7 : Clinical assessment of Dryness score for both Benchmark and Glycolysine cleanser (Rhamnolysine based cleanser) in the Atopic prone skin group, at different time points.

Clinical grading (softness, smoothness, dullness, overall skin quality / health): Glycolysine Cleanser demonstrated significantly better improvement in Overall Skin Quality / Health Clinical Grading at 24hours compared to Benchmark and at 8 hours (p=0.032).
Other assessments: Both test formulas demonstrated significant improvement in subjective tolerance – itching throughout study duration (Timm, 8h, 24h, W2, W'). 93% participants shared immediate confidence to use Glycolysine cleanser despite their reactive skin.

d) Microbiological data

Baseline Observations: At baseline, the total bacterial load was found to be significantly higher in the Atopic Dermatitis (**AD**) group compared to the Cosmetic Intolerance Syndrome (**CIS**) group (Fig. 8A), indicating a notable difference in the microbial environment between these two conditions. Similarly, the *S. aureus* load was significantly elevated in the AD group compared to the CIS group (Fig. 8B), which aligns with the established role of *S. aureus* in exacerbating symptoms of atopic dermatitis. In contrast, no statistically significant differences were observed in *S. epidermidis* levels between the groups (Fig. 8C), suggesting that this commensal bacterium was uniformly distributed across the various skin conditions.

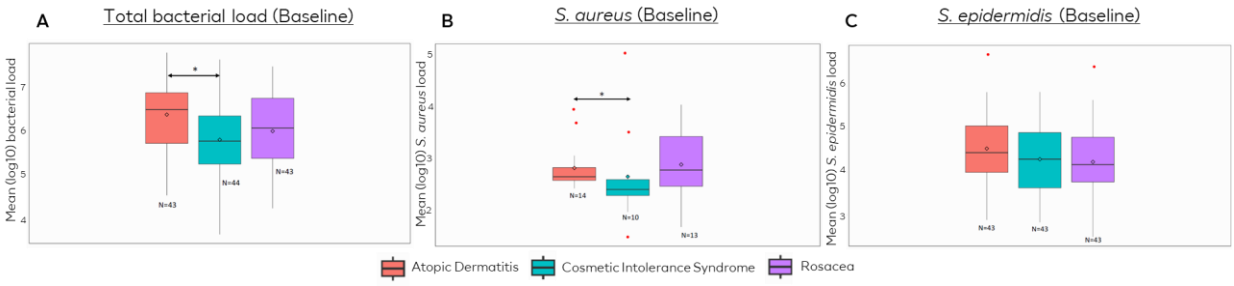


Figure 8. Bacterial loads at baseline measured by qPCR. A) Total bacterial load, B) *S. aureus* load, C) *S. epidermidis* load for the 3 different sub-groups.

S. aureus was more prevalent in the **AD** group (33%) and the Rosacea group (**R**) (31%) compared to the CIS group (23%). Notably, *S. epidermidis* was detected in all samples across all groups, underscoring its ubiquitous presence in the skin microbiome regardless of the underlying condition.

Post-Treatment Observations: Following treatment, none of the cleansers tested had a significant impact on the total bacterial load in any of the groups, including **AD**, CIS, and **R** (Figure 9). This suggests that the cleansers did not alter the overall microbial population on the skin. Similarly, no significant changes were observed in *S. epidermidis* levels after treatment (Figure 10), indicating that the cleansers were gentle and did not disrupt this beneficial commensal bacterium.

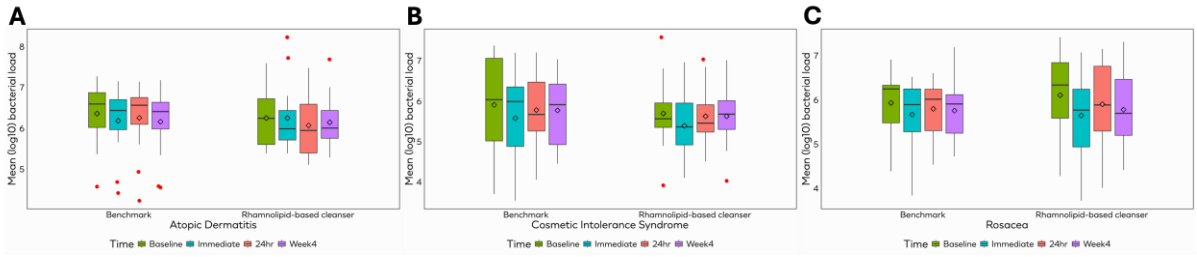


Figure 9. Impact of cleansers on bacterial loads, as measured by qPCR, under various conditions: A) Atopic Dermatitis, B) Cosmetic Intolerance Syndrome, and C) Rosacea.

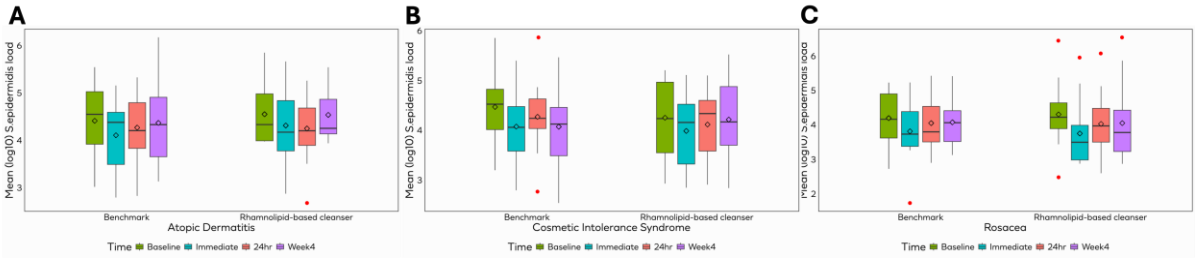


Figure 10. Impact of cleansers on *S.epidermidis* loads, as measured by qPCR, under various conditions: A) Atopic Dermatitis, B) Cosmetic Intolerance Syndrome, and C) Rosacea.

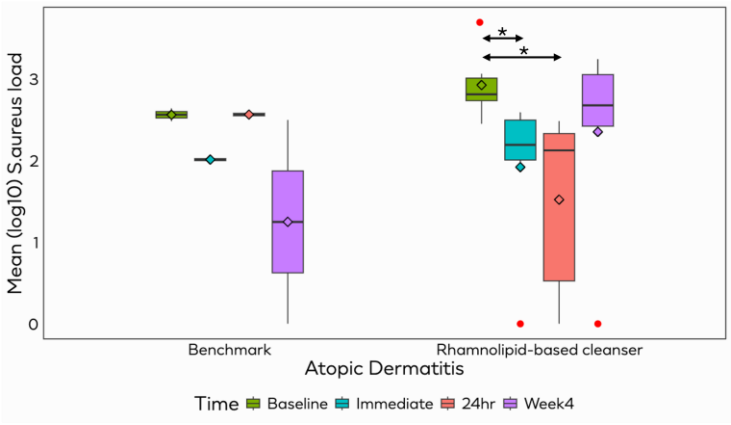


Figure 11. Influence of Benchmark and Glycolysine (Rhamnolipid based) cleansers on *S. aureus* loads, assessed by qPCR, in the Atopic Dermatitis group at different time points.

In the **AD** group, however, a significant decrease in *S. aureus* levels was observed immediately after the application of the Glycolysine cleanser (rhamnolipid-based autofoam cleanser), with the reduction measuring approximately 1 log unit. This decrease in S. aureus load was sustained for 24 hours, with a further reduction of 0.4 log unit (Figure 11). By week 4, the mean *S. aureus* levels were lower compared to baseline, although this reduction was not statistically significant. These findings suggest that Glycolysine cleanser (rhamnolipid-based autofoam cleanser) may have a cumulative effect in reducing *S. aureus* levels over time.

4. Discussion

Across all conditions studied—AD, CIS, and Rosacea—none of the cleansers demonstrated a significant impact on total bacterial load or *S. epidermidis* levels. This indicates that the cleansers were microbiome-friendly and did not disrupt the overall skin microbial balance. An increased performance in many clinical endpoints is seen for Glycolysine cleanser versus Benchmark in the case of atopic dermatitis prone skin group, where the *S aureus* load is the higher and where this load is decreased. This could be correlated with the anti-adhesion mechanism of action of glycolipid versus pathogen bacteria *S. aureus.* This anti-adhesion property has been measured also for the Glycolysine formula and not for the Benchmark formula (data not shown). Since atopic dermatitis has the greatest barrier defect, it is understandable that the Glycolysine autofoam cleanser would have most profoundly improved this condition.

5. Conclusion

This study demonstrates the performance of targeted microbiome benefits of glycolipid biosurfactant, used in a cleansing routine, for reactive skin, particularly in case of atopic dermatitis prone skin.

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