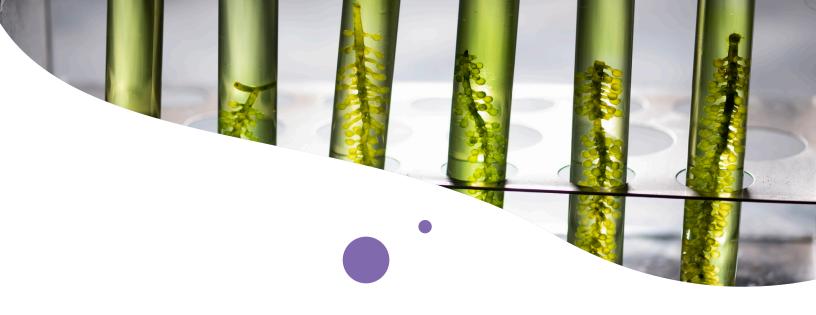


BioReNuva® WHITE PAPER Summer 2024

Enhancing Sebum Control and Skin Moisturization The Role of Sophorolipids (Glycolipids) in Treating Compromised Skin

Table of Contents Whitepaper.

Concept Introduction	03
Sebum Control	04-05
Overview	04
Mechanism of Action	05
Moisturization Improvement	06-07
Overview	06
Mechanism of Action	07
Conclusions	08
Applications Concepts	09
Figures and Charts	10-11



01. Concept Introduction Leveraging Biotechnology

01

Sophorolipids, a class of glycolipid biosurfactants, are pivotal ingredients in advanced beauty and personal care products. These naturally occurring compounds exhibit extraordinary efficacy in managing sebum production and enhancing skin moisturization, making them indispensable in facial cleansers, anti-acne treatments, moisturizers, scalp and hair care products, and hand soaps.

The Science

Scientific research underscores the multifaceted benefits of sophorolipids. Their ability to reduce and disperse sebum is achieved through superior surface activity and emulsification properties. This ensures balanced sebum distribution, preventing buildup that

leads to acne and other skin issues. Additionally, sophorolipids reinforce the skin's barrier function, significantly reducing transepidermal water loss and enhancing hydration.

Market Ready

The dual action of sebum control and moisturization makes sophorolipids exceptionally suited for a broad spectrum of beauty applications. As a gentle yet effective biosurfactant, they offer a natural solution that aligns with the growing consumer demand for safer, sustainable cosmetic ingredients. This white paper explores the scientific mechanisms by which sophorolipids deliver these benefits, substantiating their role as key actives in modern skincare and haircare formulations.

Sebum Control Mechanism of Action

0.05 - 0.5 MIC

Measured minimum inhibitory concentration (MIC) of a range of 30-70% lactone concentrated Sophorolipids against Cutibacterium acnes (C. acnes)^{4,12}.

Sebum control is a critical aspect in dermatology and the cosmetics field, directly influencing skin health and the prevention of acne. Sebum, an oily substance produced by sebaceous glands, plays a vital role in maintaining skin hydration and providing a protective barrier against environmental aggressors. However, excessive sebum production can lead to oily skin, clogged pores, and the proliferation of acne-causing bacteria, resulting in acne outbreaks and other dermatological issues.

The challenge in skincare is to balance sebum levels to ensure the skin remains well-hydrated and protected without becoming overly oily. This balance is essential for preventing acne and maintaining overall skin health. Effective sebum control can help reduce shine, minimize pore appearance, and prevent the formation of acne lesions, making it a key target for dermatological treatments and cosmetic products.

Sophorolipids (INCI: Glycolipids), a class of glycolipids derived from yeast, have emerged as promising agents in the quest for better sebum control. Their unique properties, both in their acid and lactone states Figure 2, make them highly effective in managing sebum levels, thus enhancing skin health. This paper explores the mechanisms by which sophorolipids contribute to sebum reduction and dispersion. Specifically, it delves into their ability to reduce surface tension, their antimicrobial behavior against acne-causing bacteria, and their emulsification properties. By understanding these mechanisms, we can appreciate how sophorolipids offer a multifunctional approach to improving skin condition, making them ideal for use in various dermatological and cosmetic

Multifaceted Approach

Reduction of Surface Tension

Sophorolipids effectively reduce the surface tension of aqueous solutions from 72.8 mN/m to 30-40 mN/m ^{1,2}. This significant reduction, and corresponding low CMC, allows for faster and more efficient micelle formation improving the 1) "Roll-up" or Removal and 2) Even Distribution of lipid (sebum) across the skin or hair surface ^{Figure 1}. Ultimately preventing localized accumulation of sebum which can lead to clogged pores and acne ^{1,2}. Working effectively in both leave-in and wash off applications, sophorolipids ensure that the skin maintains an optimal level of hydration and protection.

ANTIMICROBIAL ACTIVITY

Sophorolipids exhibit antimicrobial properties that help in controlling the population of harmful bacteria on the skin ^{2 Figure 3a and 3b}. By reducing the microbial load, particularly of acne-causing bacteria, sophorolipids indirectly modulate sebum production by minimizing inflammation and the immune response that often triggers excess sebum secretion. This antimicrobial effect is crucial for maintaining overall skin health and preventing acne flare-ups ^{3 Figure 4}. The amphiphilic nature of sophorolipids is related to their antibacterial activity, which involves synergistic interactions between the sugar and the lipid moieties, leading to destabilization and rupture of pathogen membrane and cell lysis.

Emulsification Properties

The strong emulsifying capabilities of sophorolipids, with a CMC of roughly 10 mg/l¹¹, facilitate the breakdown and dispersion of sebum. This is crucial in preventing the formation of oily patches and ensuring that excess sebum is efficiently removed during cleansing routines ^{1,2}. The emulsification process helps to maintain a balanced skin microbiome by preventing the overgrowth of sebum-dependent bacteria like Propionibacterium acnes, a key contributor to acne development ^{2 Figure 7a,b,c}.

Moisturization Improvement Mechanism of Action

3.3x Increase

In skin water content improvement was shown in comparison with the placebo area with Water extraction⁹.

The skin, the body's largest organ, serves as a critical barrier protecting against environmental hazards, pathogens, and water loss. Structurally, it comprises three primary layers: the epidermis, dermis, and hypodermis. The outermost layer, the stratum corneum, is integral to skin barrier function and hydration. It consists of corneocytes embedded in a lipid matrix composed mainly of ceramides, cholesterol, and fatty acids

Sophorolipids enhance skin barrier function by integrating into the lipid matrix of the stratum corneum⁵. This integration improves lipid organization and cohesion, thereby fortifying the barrier against environmental aggressors and reducing transepidermal water loss (TEWL). Strengthening the skin barrier helps to maintain optimal hydration levels, which is crucial for healthy, resilient skin.

Furthermore, the humectant properties of sophorolipids play a critical role in skin moisturization. Their sugar-derived hydrophilic moieties attract and retain water molecules from both the environment and deeper skin layers, effectively enhancing the skin's hydration status. This moisture retention capability contributes to improved skin smoothness, suppleness, and overall appearance. Additionally, sophorolipids exhibit anti-inflammatory and antioxidant activities, which are beneficial for maintaining skin health. By mitigating inflammatory responses and oxidative stress, sophorolipids help to protect the skin from damage and support the maintenance of a healthy skin barrier. This dual action not only aids in moisture retention but also promotes a balanced and calm skin environment, reducing the risk of irritation and premature aging.

Incorporating sophorolipids can enhance product efficacy in delivering long-lasting hydration and protecting skin health. Their natural origin, coupled with multifunctional benefits, makes sophorolipids a compelling choice for modern skincare innovations aimed at improving skin barrier function, hydration, and resilience.

A Unique Structure Mimicking Biology

Enhancing Skin Barrier Function

Sophorolipids integrate into the lipid matrix of the stratum corneum, improving cohesion and integrity. This integration helps to fortify the skin barrier, thereby reducing transepidermal water loss (TEWL) and promoting moisture retention Figure 5. The ability of sophorolipids to reinforce the skin barrier is well-documented, highlighting their role in enhancing the skin's protective functions 6. Studies have shown Sophorolipids to provide up to a 3.3x increase in skin water content versus a placebo.

HUMECTANT PROPERTIES

The hydrophilic nature of the sugar moiety in sophorolipids allows these molecules to attract and retain water from the environment and deeper skin layers. This characteristic enhances the skin's hydration levels by drawing moisture to the surface, leading to improved skin suppleness and smoothness. Studies have shown that the hygroscopic properties of sugars, including those in sophorolipids, are effective in maintaining skin moisture ⁷.

Anti-inflammatory and Antioxidant Effects

Sophorolipids exhibit significant anti-inflammatory and antioxidant activities, which help in reducing skin inflammation and oxidative stress. By mitigating these factors, sophorolipids contribute to maintaining the skin's natural moisture balance and barrier function. For example, Sophoroliids were found to suppress lipopolysaccharide-induced inflammation in macrophages by significantly reducing pro-inflammatory mediators such as TNF-α, ROS, Ca2+, NO, iNOS, COX-2, and IL-6^{Figure 6}. The reduction of inflammation and oxidative damage helps in preventing disruptions to the skin barrier, thus aiding in moisture retention ⁸.



What Are Sophorolipids?

Sophorolipids are a type of glycolipid biosurfactant produced by certain yeast species, such as Candida bombicola, through microbial fermentation. They consist of a sophorose suga moiety linked to a fatty acid tail via a glycosidic bond, giving them both hydrophilic and hy drophobic properties. This amphiphilic nature, paired with a very low CMC, allows sophoroli pids to reduce surface and interfacial tension between different phases, such as oil and water making them effective in a wide range of applications in personal care products

Sophorolipids are valued for their mildness, biodegradability, and multifunctional benefits. They can enhance skin barrier function, improve hydration, and provide anti-inflammatory and antioxidant effects, making them suitable for formulations aimed at moisturizing, soothing, and protecting the skin. Their natural origin and eco-friendly production process also align with the growing demand for sustainable and biocompatible ingredients in cosmetic products.

Conclusion: The Multifaceted Benefits of Sophorolipids in Advanced Skincare

The origins of biotechnology date back to ancient civilizations where fermentation processes were used for brewing, baking, and cheese-making. Modern biotechnology, which began to take shape in the late 20th century with advancements in genetic engineering and microbiology, has revolutionized many industries, including pharmaceuticals, agriculture, and cosmetics. In the beauty care market, biotechnology has enabled the development of innovative ingredients like biosurfactants, peptides, and biopolymers, which offer superior performance and sustainability compared to traditional synthetic chemicals.

In addition to their barrier-enhancing properties, sophorolipids exhibit potent anti-inflammatory and antioxidant activities. These properties are vital in controlling various skin conditions, such as eczema, dermatitis, and acne, by reducing oxidative stress and inflammation. Their mild antimicrobial effects further aid in managing acne by inhibiting the growth of acne-causing bacteria, promoting clearer and healthier skin.

Sophorolipids also support sebum control, making them beneficial for oily and acne-prone skin. By regulating sebum production and maintaining a balanced skin environment, they help prevent the clogging of pores and the formation of acne lesions. Their gentle cleansing and moisturizing properties make them suitable for a wide range of skincare products, from cleansers and moisturizers to antiaging serums and specialized treatments for compromised skin.

Formulations Concepts Highly Flexible Systems









Moisturizing Creams and Lotions

Benefits: Enhance skin barrier function and improve hydration.

Formulations: Sophorolipids can be incorporated into moisturizing bases to improve water retention and enhance the lipid barrier, making the skin more resilient to environmental stressors.

Anti-Acne

Benefits: Reduce inflammation and combat acne-causing bacteria

Formulations: Sophorolipids anti-inflammatory properties help reduce redness and swelling, while their mild antimicrobial activity can help control the growth of acnecausing bacteria, promoting clearer skin

Cleansers and Face Washes

Benefits: Gentle cleansing with moisturizing properties.

Formulations: Use sophorolipids as mild surfactants in facial cleansers to effectively remove impurities without stripping the skin of its natural oils, leaving it hydrated and soft.

Hydrating Masks

Benefits: Intense hydration and barrier reinforcement.

Formulations: Sophorolipids can be included in hydrating masks, such as sheet masks or overnight masks, to provide a concentrated dose of moisture and improve skin barrier integrity.



Hand and Body Washes

Benefits: Moisturize while cleansing.

Formulations: Use sophorolipids in hand and body wash formulations to ensure effective cleansing with the added benefit of moisturizing and protecting the skin.



Baby Care Products

Benefits: Gentle, moisturizing protection for sensitive skin.

Formulations: Develop baby lotions, shampoos, and creams with sophorolipids to ensure gentle cleansing and moisturizing suitable for delicate infant skin.



Sunscreens

Benefits: Reduce oxidative damage from UV exposure.

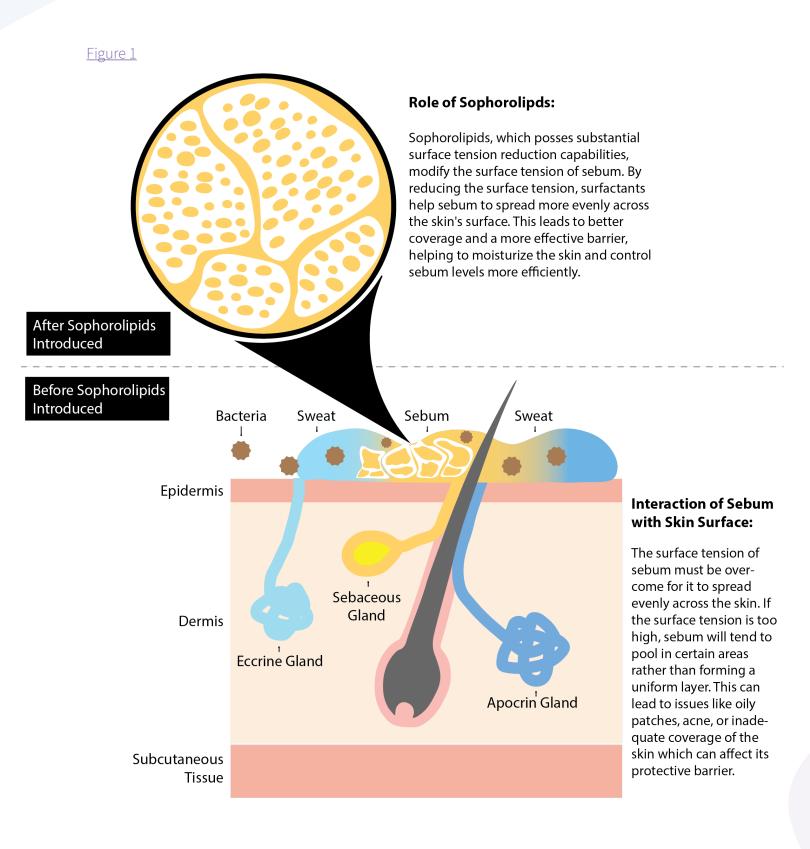
Formulations: Incorporate sophorolipids to provide additional antioxidant protection and support the skin's natural defense mechanisms against UV-induced damage.



Shaving Creams and Aftershaves

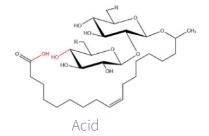
Benefits: Moisturize and soothe skin post-shave.

Formulations: Improve inshaving products to provide a smooth glide during shaving and to moisturize and soothe the skin afterward, reducing irritation and dryness

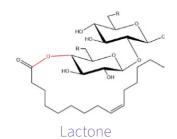


Figures and Charts

Figure 2



500



Pathogens	Sophorolipid (µg ml-1)
Escherichia coli (-)	2,000
Salmonela entérica (-)	2,000
Proteus mirabilis (-)	2,000
Staphylococcus aureus (+)	500

Sophorolipid Acid:Lactone Mix	C. acnes MIC
30:70	0.50
50:50	0.05
70:30	0.05
99:1	0.02 - 0.05

Figure 3a

Streptococcus mutans (+)

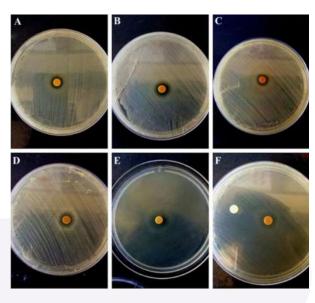


Figure 3b

(A-Escherichia coli, B-Salmonella enterica subsp. enterica, C-Proteus mirabilis) and Gram-Positive (D-Staphylococcus aureus, E-Streptococcus mutans and F-Enterococcus faecium and a white disk, control, without any antimicrobial).

Figure 4

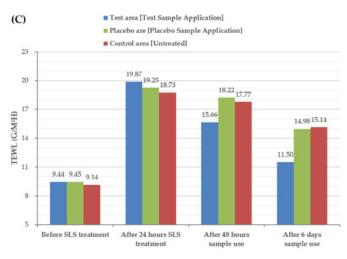


Figure 5

TEWL examination at 24 hours, 48 hours and 6 days of sample use.

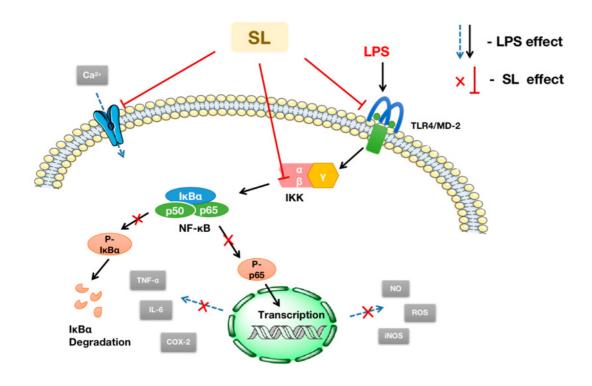


Figure 6
Schematic illustration of the anti-inflammatory action and mechanism of Sophorolipids in a macrophage RAW264.7 Cells.



Figure 7a 4 week study, n=5, to asses the ability for lactone sophorolipids to reduce acne lesions. 62.22% overall reduction recorded in this subject.



Figure 7b
Photographic analysis of two selected lesions showed a 99.93% and 99.88% reduction in size afer 4 weeks of product use.

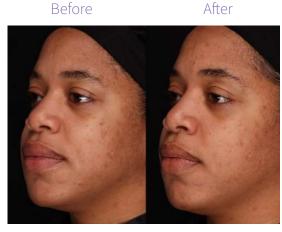


Figure 7c 4 week study, n=5, to asses the ability for lactone sophorolipids to reduce acne lesions. 47.65% overall reduction recorded in this subject.

BioReNuva

References:

- 1. Van Bogaert, I. N., Saerens, K., De Muynck, C., Develter, D., Soetaert, W., & Vandamme, E. J. (2007). Microbial production and application of sophorolipids. Applied Microbiology and Biotechnology, 76(1), 23-34. https://doi.org/10.1007/s00253-007-0988-7
- 2. Daverey, A., & Pakshirajan, K. (2009). Sophorolipids production by Candida bombicola using synthetic dairy wastewater. Bioresource Technology, 100(24), 5869-5872. https://doi.org/10.1016/j.biortech.2009.06.066
- 3. Minucelli, T., Viana, M. R. R., Borsato, D., Galdino, A. B., Cely, M. T. V., Oliveira, M. R., Baldo, C., & Celligoi, M. A. P. C. (2020). Antibacterial Activity of Sophorolipids from Candida bombicola Against Human Pathogens. *Brazilian Archives of Biology and Technology*, 63. https://doi.org/10.1590/1678-4324-2020180568
- 4. Richard D. Ashby, Jonathan A. Zerkowski, Daniel K.Y. Solaiman, Lin Shu Liu, Biopolymer scaffolds for use in delivering antimicrobial sophorolipids to the acne-causing bacterium propionibacterium acnes, New Biotechnology, Volume 28, Issue 1, 2011, Pages 24-30.
- 5. Adu, S.A.; Twigg, M.S.; Naughton, P.J.; Marchant, R.; Banat, I.M. Purified Acidic Sophorolipid Biosurfactants in Skincare Applications: An Assessment of Cytotoxic Effects in Comparison with Synthetic Surfactants Using a 3D In Vitro Human Skin Model. Fermentation 2023, 9, 985. https://doi.org/10.3390/fermentation9110985
- 6. Lee YR, Cha YJ, Jeong S, Yun SK, Nho Y, Kang S, Kim W, Son J, Kim J, Kyung S. A novel sophorolipids extraction method by yeast fermentation process for enhanced skin efficacy. Skin Res Technol. 2023 Nov;29(11):e13518. doi: 10.1111/srt.13518. PMID: 38009026; PMCID: PMC10643984.

Contact:

info@biorenuva.com 801 Barton Springs RD Austin, TX 78704

Whitepaper 2024

BioReNuva Company Series Alpha www.biorenuva.com

- 7. Adu SA, Naughton PJ, Marchant R, Banat IM. Microbial Biosurfactants in Cosmetic and Per-sonal Skincare Pharmaceutical Formulations. Pharmaceutics. 2020 Nov 16;12(11):1099. doi: 10.3390/pharmaceutics12111099. PMID: 33207832; PMCID: PMC7696787.
- 8. Xu R, Ma L, Chen T, Wang J. Sophorolipid Sup-presses LPS-Induced Inflammation in RAW264.7 Cells through the NF-kB Signaling Pathway. Molecules. 2022 Aug 8;27(15):5037. doi: 10.3390/molecules27155037. PMID: 35956987; PMCID: PMC9370320.
- 9. Lee YR, Cha YJ, Jeong S, Yun SK, Nho Y, Kang S, Kim W, Son J, Kim J, Kyung S. A novel sophoro-lipids extraction method by yeast fermentation process for enhanced skin efficacy. Skin Res Technol. 2023 Nov;29(11):e13518. doi: 10.1111/srt.13518. PMID: 38009026; PMCID: PMC10643984.
- 10. Kim, K., Yoo, D., Kim, Y., Lee, B., Shin, D. & Kim, E.-K. (2002) Characteristics of sophorolipid as an antimicrobial agent. Journal of Microbiology and Biotechnology, 12, 235–241.
- 11. Otto RT, Daniel HJ, Pekin G, Müller-Decker K, Fürstenberger G, Reuss M, Syldatk C. Production of sophorolipids from whey. II. Product composition, surface active properties, cytotox-icity and stability against hydrolases by enzy-matic treatment. Appl Microbiol Biotechnol. 1999 Oct;52(4):495-501. doi: 10.1007/s002530051551. PMID: 10570796.
- 12. BioReNuva LLC Internal Study | Review of MIC data for C. acnes at varying lactone concentra-tions (30-99%).