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Balancing the Skin Microbiome: Postbiotics in Acne Control

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1. Introduction:

Human skin is a complex ecosystem that hosts a rich diversity of microorganisms, which coexist symbiotically with the host [1]. This population of microorganisms, known as the skin microbiome, plays a crucial role in maintaining skin homeostasis by acting as a barrier against pathogens, modulating the immune response, and contributing to skin integrity [2,3]. However, imbalances in this microbial community, a phenomenon known as dysbiosis, are associated with various dermatological conditions, including acne vulgaris [4,5].

Acne is a chronic inflammatory skin condition that predominantly affects adolescents and young adults, although it can persist or emerge at other ages. It is characterized by comedones, papules, pustules, and in more severe cases, nodules and scarring. The etiology of acne is multifactorial, involving sebaceous hypersecretion, follicular hyperkeratinization, inflammation, and the exacerbated colonization of the pilosebaceous follicle by the bacterium *Cutibacterium acnes* (previously known as *Propionibacterium acnes*) [6,7]. Under dysbiotic conditions, this bacterium becomes dominant and induces exacerbated inflammatory responses, contributing significantly to the pathogenesis of the disease [8].

Conventional acne therapies include the use of topical and systemic antibiotics, retinoids, and anti-androgenic agents. However, these approaches have limitations such as side effects, bacterial resistance, and negative impacts on the skin microbiome [9,10]. Consequently, new strategies for acne treatment, focused on restoring microbial balance with lower resistance risks and higher biocompatibility have gained prominence in recent years.

In this context, postbiotics—bioactive metabolites produced by probiotic microorganisms during fermentation, without living cells—have emerged as a promising alternative. Studies indicate that postbiotics can modulate the inflammatory response, improve the skin barrier, and inhibit the growth of pathogens, thus contributing to the balance of the skin microbiome [11,12]. Additionally, they offer advantages over conventional treatments, such as a lower risk of inducing bacterial resistance and fewer side effects [13]. Another benefit is that postbiotics,

unlike live probiotics, do not require cell viability to exert beneficial effects, making them more stable, safer, and easier to incorporate into topical formulations.

Among the microorganisms of interest for postbiotic production, *Lacticaseibacillus rhamnosus* is particularly notable [14]. Although *L. rhamnosus* is widely studied in the context of gut health, its topical application in dermatological treatments, especially for acne control, represents an emerging and still underexplored area. Growing evidence highlights its potential: derivatives of *L. rhamnosus* have demonstrated the ability to improve skin barrier function [15] and exhibit antimicrobial activity against bacteria associated with skin diseases, such as *C. acnes* [16]. Moreover, research on other species of the Lactobacillaceae family, such as *Lactiplantibacillus plantarum*, has confirmed the efficacy of topical formulations containing postbiotics in reducing acne lesions, reinforcing the relevance of this approach [17].

Therefore, the present study aimed to evaluate the efficacy of a postbiotic ingredient, derived from the fermentation of *L. rhamnosus*, in inhibiting *C. acnes*, reducing inflammation, and controlling sebum production. The investigation intends to explore the potential of this product as a safe and effective alternative for acne treatment, contributing to the advancement of dermocosmetic strategies aligned with microbiota preservation, sustainability, and biotechnological innovation.

2. Materials and Methods:

2.1 Evaluation of Anti-Inflammatory Potential and Sebum Control (*in vitro*):

For the assay, human keratinocyte cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium) and incubated at 37°C with 5% CO_2 under aseptic conditions. The tested sample, a postbiotic derived from the fermentation of *L. rhamnosus*, was diluted to a final concentration of 1% in the culture medium. The control group consisted of cells exposed only to the culture medium, without the addition of the tested sample.

The cells were incubated with the experimental solutions for 48 hours. After this period, messenger RNA (mRNA) was extracted using Trizol® reagent, and its purity and concentration were determined by spectrophotometry. cDNA was synthesized through a reverse transcriptase reaction, followed by the quantification of gene expression of IL-1, IL-8, and the enzyme 5-α-reductase by RT-qPCR (Real-Time Reverse Transcription Polymerase Chain Reaction). The GAPDH gene was used as an endogenous control for normalization of relative expression. Relative expression data were analyzed using the 2^(-ΔΔCt) method. Statistical analyses were performed using GraphPad Prism® software with a Student's t-test for comparison between groups, considering statistical significance at $p < 0.05$.

2.2 Evaluation of Antibacterial Activity by the Disk Diffusion Method:

Antibacterial activity was initially evaluated using the disk diffusion method. *C. acnes* (ATCC 6919) was cultivated in BHI (Brain Heart Infusion) medium at 30°C under anaerobic conditions. After 72 hours of growth, 500 µL of this culture was plated onto blood agar plates.

Afterward, sterile filter paper disks, 6 mm in diameter, were placed on the surface of the seeded agar, and 15 µL of the tested product (postbiotic derived from the fermentation of *L. rhamnosus*) were applied to each disk, in triplicate. As a negative control, 15 µL of the culture medium used in the postbiotic production, without bacterial fermentation, was applied. The plates were incubated at 30°C under anaerobic conditions for 15 days. After this period, the diameters of the inhibition halos formed around the disks were measured.

2.3 Determination of Minimum Inhibitory Concentration (MIC):

To determine the minimum inhibitory concentration (MIC) of the investigated product against *C. acnes* (ATCC 6919), a serial dilution of the product was performed in sterile 96-well microplates. Then, 100 µL of the bacterial culture of *C. acnes*, previously cultivated in Brain Heart Infusion (BHI) medium and adjusted to a final concentration of 5×10^5 CFU/mL, was added. Bacterial growth was monitored by measuring the optical density at 600nm using the Varioskan LUX multimode microplate reader (Thermo Fisher Scientific Inc., Waltham, MA, USA). The MIC was defined as the lowest concentration of the product capable of inhibiting visible growth of *C. acnes* after incubation at 37°C for 72 hours under anaerobic conditions. As a positive control for inhibition, ampicillin (1 µg/mL) was used. All assays were performed in triplicate.

2.4 Antioxidant Activity:

The percentage of antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, following a previously described methodology [18], with some adaptations. A DPPH solution (32 µg/mL) was prepared in ethanol and protected from light. The positive control for the test, composed of a quercetin solution in ethanol, was prepared at a concentration of 2 mg/mL.

Sample preparation was carried out as follows: for the negative control only the DPPH solution was used; for the reaction blank only ethanol was used; for analysis of the investigated product, 900 µL of the DPPH solution and 100 µL of the product were added. Additionally, sample blanks were prepared using 100 µL of the product and 900 µL of ethanol to eliminate interference from the color of the active compound on the absorbance reading. The mixtures were kept in the dark for 30 minutes and then absorbance was read at 517 nm using a UV/Vis spectrophotometer. The percentage of antioxidant activity was calculated using the following equation:

$$\%\ Antioxidant\ activity = \frac{(Absorbance_{negative\ control} - (Absorbance_{Sample} - Absorbance_{Sample\ blank}))}{Absorbance_{negative\ control}} \times 100$$

3. Results:

3.1 Evaluation of Anti-Inflammatory Potential and Sebum Control (*in vitro*):

The *in vitro* assay demonstrated that the postbiotic was able to reduce the gene expression of the enzyme 5-α-reductase by 35.8% compared to the control group, as shown in Figure 1. The enzyme 5-α-reductase is responsible for converting testosterone into dihydrotestosterone (DHT), one of the main factors that stimulate excessive sebum production. Inhibition of this enzyme by the investigated postbiotic indicates relevant potential in regulating skin oiliness, a key factor in acne treatment.

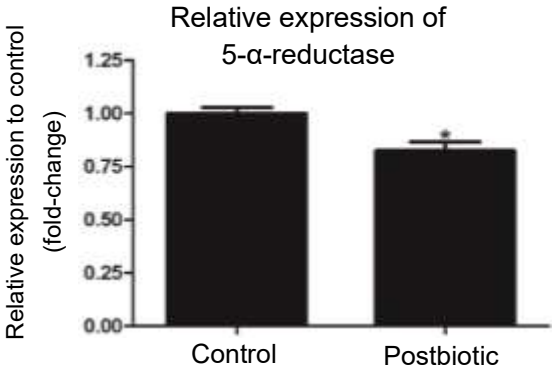


Figure 1. The postbiotic reduced 5-α-reductase gene expression by 35.8% compared to the control group (*p < 0.05).

In addition, the postbiotic showed anti-inflammatory activity, evidenced by the reduction of pro-inflammatory markers IL-1 and IL-8 in human keratinocytes cells. The product reduced IL-1 expression by 66.8% and IL-8 expression by 59.3%, as demonstrated in Figure 2. These results suggest that the ingredient may modulate the exacerbated inflammatory response that characterizes acne lesions.

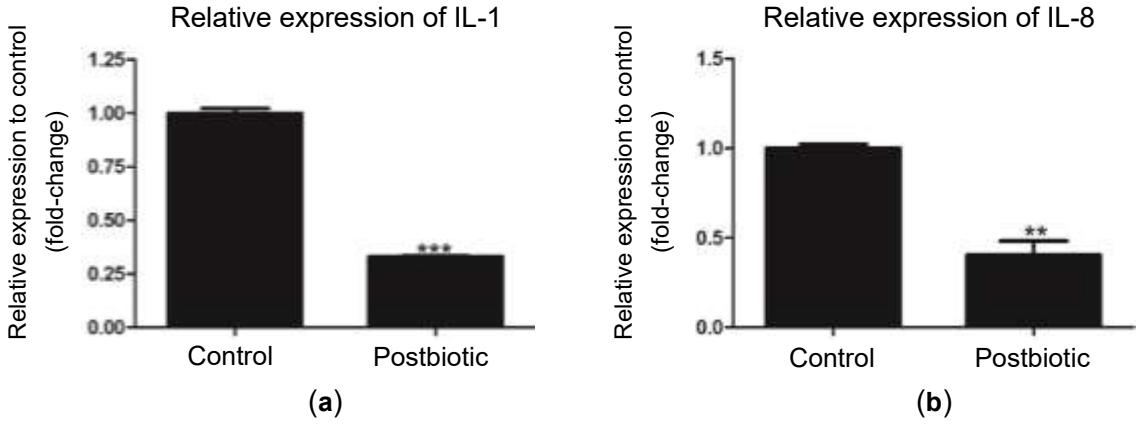
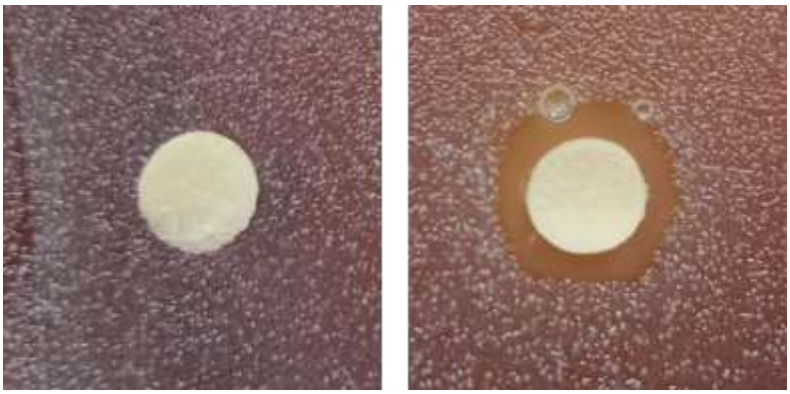


Figure 2. Relative gene expression analysis of pro-inflammatory cytokines IL-1 and IL-8. (a) The postbiotic decreased IL-1 expression by 66.8% compared to the control group (***p < 0.001). (b) The postbiotic decreased IL-8 expression by 59.3% compared to the control group (**p < 0.01).

3.2 Evaluation of Antibacterial Activity by the Disk Diffusion Method:

The results, presented in Figure 3, demonstrate the formation of an inhibition halo with an average diameter of 8.97 mm, evidencing the antibacterial activity of the tested postbiotic against *C. acnes*, as it prevented bacterial growth around the disc impregnated with the product.



(**a**) (**b**)

Figure 3. Antibacterial activity evaluation against *C. acnes* using the disk diffusion method. (a) Negative control, showing bacterial growth up to the edge of the disk with no inhibition halo. (b) Disk containing the investigated postbiotic, showing a clear inhibition halo around the disk, indicating bacterial growth inhibition.

3.3 Determination of Minimum Inhibitory Concentration (MIC)

In the test to determine the minimum inhibitory concentration (MIC) of the postbiotic against *C. acnes* (ATCC 6919), it was observed that concentrations equal to or higher than 6.25% completely inhibited bacterial growth over 72 hours, as determined by optical density measurement (Figure 4).

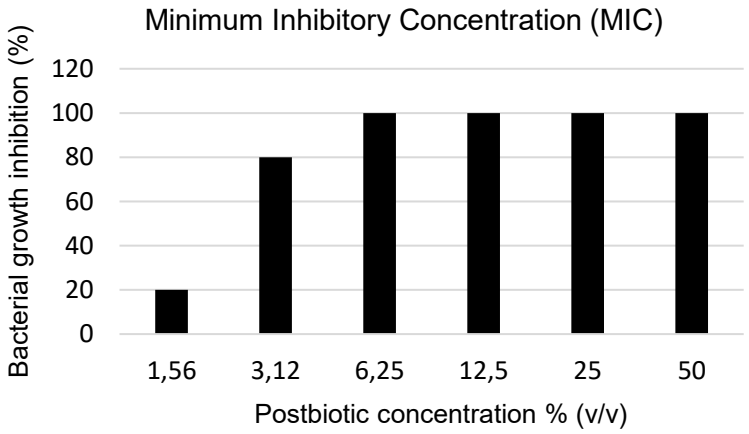


Figure 4. MIC determination of the postbiotic against *C. acnes* (ATCC 6919), indicating total growth inhibition at concentrations equal to or greater than 6.25%.

3.4 Antioxidant Activity:

The antioxidant activity test showed that the postbiotic exhibited an antioxidant capacity of 92%, surpassing the positive control, represented by quercetin, which reached 79%. This result demonstrates the strong potential of the postbiotic in neutralizing free radicals, highlighting its effectiveness as an antioxidant agent.

4. Discussion:

The results obtained in this study indicate that the evaluated postbiotic acts on multiple aspects, targeting the main mechanisms associated with the development of acne: excessive sebum production, proliferation of *C. acnes*, inflammation, and cutaneous dysbiosis. These findings support the growing literature highlighting the role of metabolites derived from beneficial microorganisms in maintaining skin health [19].

The postbiotic's ability to inhibit the enzyme 5-α-reductase is particularly significant, as this enzyme is directly linked to increased sebum production, which creates a favorable environment for the proliferation of *C. acnes* [20]. By acting on this mechanism, the ingredient demonstrates not only a palliative effect but also a preventive potential, intervening in the etiological factors of acne from the beginning.

The anti-inflammatory action observed is also very important. Acne is considered a chronic inflammatory disease, and immunological processes play a central role in the formation and worsening of lesions [21]. The significant reduction of pro-inflammatory cytokines, particularly IL-1 and IL-8 [22], in keratinocytes treated with the postbiotic reinforces its potential as a skin-soothing agent, which may contribute to clinical improvement in inflammatory acne.

Regarding its antimicrobial activity, the effects against *C. acnes* are a notable advantage compared to conventional treatments, such as broad-spectrum topical antibiotics, which are often associated with the induction of bacterial resistance and disruption of the skin microbiome [9]. Therefore, the use of postbiotics represents a more ecological and functional treatment approach, aligned with current trends that value the microbial integrity of the skin.

As for its antioxidant activity, the results indicate that the active compound has the potential to reduce oxidative stress. This type of stress alters the microenvironment of the pilosebaceous unit, creating favorable conditions for the growth of anaerobic bacteria such as *C. acnes*, and inducing the activation of inflammatory transcription factors, one of the initial events in acne development [23]. Due to its antioxidant action, the postbiotic helps neutralize these processes, preventing the inflammatory cascade associated with acne pathogenesis.

It is important to note that, despite the promising results, further studies are needed, including clinical trials in human volunteers, to validate the efficacy, safety and applicability of the postbiotic across different skin types and acne severities. Personalized formulations and combination with other dermocosmetic actives may also enhance its effects.

5. Conclusion:

The data obtained throughout this study demonstrate that the evaluated postbiotic has several functional properties for acne control, acting multifactorially on the main mechanisms involved in the pathogenesis of this skin condition and positioning this ingredient as an innovative alternative to traditional approaches, which are often limited by adverse effects, bacterial resistance, and negative impacts on the skin microbiota.

Moreover, the fact that the ingredient is derived from beneficial microorganisms cultured in the lab, without requiring direct extraction from natural resources, gives it a sustainable advantage. This not only reduces the environmental impact associated with its production but also contributes to the development of safer cosmetics, particularly in a context of growing demand for products that protect both skin and environmental integrity. Based on the results presented, it can be concluded that postbiotics represent a new generation of active ingredients with significant potential for use in dermocosmetics aimed at the prevention and treatment of acne.

References:

1. Byrd, A. L., Belkaid, Y., & Segre, J. A. (2018). The human skin microbiome. Nature Reviews Microbiology, 16(3), 143–155. https://doi.org/10.1038/nrmicro.2017.157

2. Belkaid, Y., & Tamoutounour, S. (2016). The influence of skin microorganisms on cutaneous immunity. Nature Reviews Immunology, 16(6), 353–366. https://doi.org/10.1038/nri.2016.48

3. 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

4. O'Neill, C. A., & Gallo, R. L. (2018). Host-microbiome interactions and recent progress into understanding the biology of acne vulgaris. Microbiome, 6, 177. https://doi.org/10.1186/s40168-018-0558-5

5. Dreno, B., et al. (2020). The skin microbiome: A new actor in inflammatory acne. American Journal of Clinical Dermatology, 21(Suppl 1), 18–24. https://doi.org/10.1007/s40257-020-00531-1

6. Costa, A., Alchorne, M. M. A., & Goldschmidt, M. C. B. (2008). Fatores etiopatogênicos da acne vulgar. Anais Brasileiros de Dermatologia, 83(5), 451–459. https://doi.org/10.1590/S0365-05962008000500010

7. Dagnelie, M. A., Corvec, S., Saint-Jean, M., & Khammari, A. (2022). *Cutibacterium acnes* and acne vulgaris: A brief look at the latest updates. Journal of the European Academy of Dermatology and Venereology, 36(5), 732–740. https://doi.org/10.1111/jdv.15043

8. Fitz-Gibbon, S., Tomida, S., Chiu, B. H., et al. (2013). *Propionibacterium acnes* strain populations in the human skin microbiome associated with acne. Journal of Investigative Dermatology, 133(9), 2152–2160. https://doi.org/10.1038/jid.2013.21

9. Lam, M., Hu, A., Fleming, P., & Lynde, C. W. (2021). The Impact of Acne Treatment on Skin Bacterial Microbiota: A Systematic Review. Journal of Cutaneous Medicine and Surgery, 26(1), 93–97. https://doi.org/10.1177/12034754211037994

10. Walsh, T. R., Efthimiou, J., & Dréno, B. (2016). Systematic review of antibiotic resistance in acne: An increasing topical and oral threat. The Lancet Infectious Diseases, 16(3), e23–e33. https://doi.org/10.1016/S1473-3099(15)00527-7

11. Dinić, M. et al. (2024). Postbiotic lactobacilli induce cutaneous antimicrobial response and restore the barrier to inhibit the intracellular invasion of *Staphylococcus aureus* in vitro and ex vivo. *The FASEB Journal*. https://doi.org/10.1096/fj.202400054RR

12. Prajapati, S. K., Lekkala, L., Yadav, D., Jain, S., & Yadav, H. (2024). Microbiome and postbiotics in skin health. Biomedicines, 13(4), 791. https://doi.org/10.3390/biomedicines13040791

13. Majeed, M., Majeed, S., Nagabhushanam, K., Mundkur, L., & Neelakantan, S. (2020). Novel Topical Application of a Postbiotic, LactoSporin®, in Mild to Moderate Acne: A Randomized, Comparative Clinical Study to Evaluate its Efficacy, Tolerability and Safety. Cosmetics, 7(3), 70. https://doi.org/10.3390/cosmetics7030070

14. Ye, H., Liu, X., Guan, K., Ma, Y., Liu, R., Liu, Y., Lv, X., Wang, R., & Li, Q. (2024). Therapeutic potential of *Lacticaseibacillus rhamnosus* grx10 and its derived postbiotic through gut microbiota and MAPK/MLCK/MLC pathway-mediated intestinal barrier repairment in ulcerative colitis. Journal of Food Science, 89(12), 10035–10052. https://doi.org/10.1111/1750-3841.17550

15. Lai, Y., et al. (2019). Lysates of a Probiotic, *Lactobacillus rhamnosus*, Can Improve Skin Barrier Function in a Reconstructed Human Epidermis Model. International Journal of Molecular Sciences. https://doi.org/10.3390/ijms20174289

16. Xie, Y., et al. (2022). Antibacterial activity and mechanism of cell-free supernatants of Lacticaseibacillus paracasei against *Propionibacterium acnes*. Frontiers in Microbiology. https://doi.org/10.1016/j.micpath.2024.106598

17. Wong, J., et al. (2023). Development of Novel Topical Anti-Acne Cream Containing Postbiotics for Mild-to-Moderate Acne: An Observational Study to Evaluate Its Efficacy. Clinical, Cosmetic and Investigational Dermatology. https://doi.org/10.4103/ijd.ijd_655_22

18. Pires, J., Torres, P. B., Santos, D. Y. A. C. dos, & Chow, F. (2017). Ensaio em microplaca do potencial antioxidante através do método de sequestro do radical livre DPPH para extratos de algas. Instituto de Biociências, Universidade de São Paulo. ISBN 978-85-85658-71-7. https://doi.org/10.13140/RG.2.2.27450.08640

19. Guéniche, A., Philippe, D., Bastien, P., Blum, S., Buyukpamukcu, E., & Castiel-Higounenc, I. (2023). Cosmeceuticals: A review of clinical studies claiming to contain specific, well-characterized strains of probiotics or postbiotics. Nutrients, 16(15), 2526. https://doi.org/10.3390/nu16152526

20. Escamilla-Cruz, M., Magaña, M., Escandón-Perez, S., & Bello-Chavolla, O. Y. (2023). Use of 5-alpha reductase inhibitors in dermatology: A narrative review. Dermatology and Therapy, 13(4), 974-984. https://doi.org/10.1007/s13555-023-00974-4

21. Tanghetti, E. A. (2023). The Role of Inflammation in the Pathology of Acne.

22. Singh, S., Khurana, A., & Chitkara, A. (2023). Evaluation of serum levels of interleukins 6, 8, 17, and 22 in acne vulgaris: A cross-sectional study. Indian Journal of Dermatology, 68(1), 19-24. https://doi.org/10.4103/ijd.ijd_786_21

23. Wong, A. (2016). Oxidative Stress in Acne Vulgaris. Clinical Dermatology & Therapy, 3(1), 1–6. https://doi.org/10.24966/CDT-8771/100020