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ORIGINAL ARTICLE

Topical *Lactiplantibacillus plantarum* LB244R® ointment alleviates skin aging: An exploratory trial

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Abstract

Background: The skin is of vital importance for health and well-being. As people age, the skin undergoes visual and morphological changes such as wrinkling, loss of elasticity, increased pigmentation, and decreased cell turnover. This is not only visually unappealing to many but can also pose health issues.

Aim: In this study, a probiotic ointment (PO) containing live lactic acid bacteria (LAB) (*Lactiplantibacillus plantarum* LB244R®) was investigated for its ability to alleviate symptoms of skin aging in an exploratory clinical trial.

Methods: The PO was applied twice daily for 56 days by 21 subjects. Anti-aging efficacy was evaluated by skin ultrasonography, skin biomechanical properties, skin hydration, and clinical evaluations at day 0, 28, and 56.

Results: Sub-epidermal low echogenic band thickness decreased (0.261 ± 0.069 mm to 0.247 ± 0.055 mm) after 56 days. Dermal density increased (324.689 ± 57.506 pixel/mm^2 to 367.831 ± 75.790 pixel/mm^2). Skin hydration increased (34.1 ± 6.9 to 51.3 ± 10.0 AU). Additionally, skin firmness increased, as shown by decreasing values (0.264 ± 0.038 to 0.228 ± 0.037 mm). Skin elasticity increased (0.578 ± 0.045 to 0.618 ± 0.044). Trans-epidermal water loss decreased (9.1 ± 2.0 g/h/m^2 to 8.5 ± 1.3). All clinical evaluations, Crow's feet, spot score, smoothness score, and complexion radiance, were improved.

Conclusion: The PO improved all measured parameters with statistical significance after 56 days of application, clearly demonstrating the potential of the PO as an anti-aging agent and reaffirming the potential of topical probiotic LAB. Future studies need to elucidate the mode of action of anti-aging effects by probiotics, but at present time, this study paves the way for the use of probiotic LAB topically to alleviate aging of the skin.

1 | INTRODUCTION

Aging, the process of gradual decline whilst growing older, is potentially the most common all-encompassing reality, affecting everyone. One of the most visually prominent results of aging can be seen on the skin. The skin loses its firmness and elasticity, resulting in sagging, more apparent pores, and wrinkles. Additionally, the turnover number of cells in the skin decreases with the skin becoming less radiant, rougher in appearance, and drier as a result of reduced sebocyte activity, with an increasing number of pigment spots.

Aging of the skin can be considered on several levels such as phenotypically (as described above), histologically, and/or molecularly.

Histologically, the junction between the dermis and epidermis becomes flattened and melanocyte density decreases. The dermis thickness decreases, accompanied by a decrease in both vascularization and the number of mast cells and fibroblasts.[1] The reduced elasticity and firmness of the skin are a result of changes in the extracellular matrix (ECM) of the dermis, such as altered production and location of the fibers collagen and elastan.[1] The glandular activity also decreases, resulting in less sebum and subsequently more dry skin. Cellularly, aging occurs due to the accumulation of senescent cells, change in cell cycles and accumulation of free radicals/oxidative stress, and changes in glycation patterns, specifically in that of collagen.[2]

The above-mentioned changes occur due to intrinsic and extrinsic factors, where intrinsic factors include chronological and genetical factors, whereas extrinsic factors are attributed to environmental effects, such as sun (termed photoaging), pollution exposure, diet, cosmetics, and smoking. Especially sun exposure can result in the generation of reactive oxygen species (ROS) and free radicals ending with oxidative stress and subsequent cellular damage.[1] Additionally, a recent study by Howard et al. found that the diversity of the skin microbiome increased in all sites sampled with age and that the abundance of *Lactobacillus* and *Cutibacterium* decreased,[3] indicating that the skin microbiome may also play a role in skin aging, albeit causality remains undetermined. To summarize, aging occurs due to a wide variety of factors, which intricately interact and affect each other.

These changes are not only cosmetically displeasing to some but can result in protective barrier issues and subsequent medical issues. With the increasing age of the population, especially in the westernized world, there is a huge cosmetic and economic interest in reversing/preventing the appearance of aging signs.[2]

One promising strategy to prevent as well as reverse the signs of aging includes the use of probiotics. Probiotics are defined by WHO and FAO as "Live microorganisms, which, when administered in adequate amounts, provide a benefit to the host".[4] Probiotics can be applied orally or topically, both of which have previously shown ability to positively modulate skin and skin diseases.[5,6] Certain probiotic strains were found to restore pH of the skin, lessen oxidative stress, attenuate photoaging, promote skin barrier function and reduce the appearance of wrinkles.[5,6]

Lactic acid bacteria (LAB) are commonly used in probiotics and have been widely studied in human applications, albeit the topical use of LAB remains in its infancy as compared to the orally applied probiotics.

Topical application of certain probiotics has proven ability to affect/modulate dysbiotic microbiomes and thereby affect disease phenotypes. Currently, diseases in which probiotics can have a positive effect include psoriasis, atopic dermatitis, acne vulgaris, seborrheic dermatitis, and rosacea.[7] *Lactiplantibacillus plantarum* LB244R® was originally selected for topical use due to its antibacterial activity toward *Staphylococcus aureus* clonal type 1 and its ability to positively alleviate symptoms of atopic skin but has since been suspected to also positively influence aging skin.[7]

In this present study, an exploratory trial was conducted to investigate the anti-aging potential of a cosmetic probiotic ointment (PO) containing live *Lactiplantibacillus plantarum* LB244R® for topical application. The trial was run for 56 days to determine consumer acceptability as well as efficacy, where specific skin aging parameters were measured three times throughout the trial (day 0, day 28, and day 56).

2 | MATERIALS AND METHODS

2.1 | Investigational product

The investigational product was a probiotic oil-based ointment formulation (PO) of 30 mL with live LAB (*Lactiplantibacillus plantarum* LB244R®) at a concentration of 1×10^9 CFU/g. Ointments are defined as topical formulations which are oil-based and have a semi-solid, greasy texture. The product has a shelf-life of approximately 24 months when kept refrigerated. No preservatives are necessary as it is an entirely oil-based formulation.

Ingredients in the PO include, as listed on the international nomenclature of cosmetic ingredients (INCI); *Butyrospermum parkii* butter [sheabutter, Naturasoft organic Shea - refined] (Natura-Tec), *Brassica campestris* seed oil [Rapeseed oil refined organic DE öko-001] (Gustavheess), *Simmondsia chinensis* seed oil [Jojoba oil - refined] (Natura-tec), Hydrogenated olive oil [OC Wax] (Natura-Tec), *Helianthus annuus* hybrid oil [sunflower hybrid oil, Bio-sonnenblumenöl HO deso] (Bressmer), *Lactiplantibacillus plantarum* LB244R® [Live probiotic] (Lactobio), tocopherol [Vitamin E, Dermofeel Toco 70 non-GMO] (Drstraetmans), *Helianthus annuus* seed oil [Sunflower seed oil] (Bressmer).

2.2 | Clinical trial study design

The trial was designed as a single-center study of a topical ointment. The trial was conducted in accordance with the Declaration of Helsinki principles. The study design was reviewed by an internal review board (opinion no 8397/2022) and approved by the Independent Ethics Committee for human application. The study was conducted in the spirit of good clinical practice guidelines and general principles of law 46/2004 of August 19th. Informed consent from all subjects was obtained prior to the study. The study was conducted from the 1st of June 2022 to the 1st of August 2022.

In total, 23 subjects were included. Subjects were postmenopausal females between 49 and 62 years with "normal" or mixed/dry to dry skin types. The subjects had to meet the following inclusion criteria: Fitzpatrick skin phototype between II and IV with the presence of skin aging,[8] such as wrinkles, fine lines, and pigmented spots, with at least grade 2 up to grade 5 on the Bazin scale[9] (Table 1).

TABLE 1 Trial demographics.

Demographic Data		Skin		Subjects	
Number	23 (100%)	*Skin reactivity*		Included	23 (100%)
Mean age	56.6	Sensitive	14 (60.9%)	Completed	21 (91.3%)
Age min.	49	Normal	9 (39.1%)	Dropouts	2 (8.7%)
Age max.	62	*Skin condition*			
Phototype II	4 (17.4%)	Normal	9 (39.1%)		
Phototype III	16 (69.6%)	Mixed/Dry	10 (43.5%)		
Phototype IV	3 (13.0%)	Dry	4 (17.4%)		

Exclusion criteria included: cutaneous marks interfering with assessment, allergies to ingredients in the product, a history of skin cancer, changed hormonal treatment, prior Vitamin A or other anti-aging treatments (e.g., Botox or other injectables), or a forecast of intensive sun exposure during the test period. For all exclusion criteria see Appendix S1.

Subjects were instructed to apply the PO two times daily (morning and evening) on clean facial skin and massage until absorption for 56 consecutive days. The subjects were each given 2 POs (30 mL). The subjects were assessed by dermatologists prior to treatment initiation (Day 0, D0), 28 days after treatment initiation (D28 ± 2 days), and 56 days after treatment initiation (D56 ± 3 days). All evaluations were performed in a controlled environment after an acclimatization process of at least 15 minutes in a fully controlled and acclimatized room (temperature of 21 ± 2°C and relative humidity of 55 ± 10%).

2.3 | Product compatibility, safety, and acceptability assessment

Compatibility was assessed by the sensation of any discomfort described by the subjects and the visible reactions of irritation determined by dermatologists or trained technicians. This examination was performed visually under standard daylight. The main discomfort signs assessed included erythema, edema, formation of vesicles, formation of papules, scabs, dryness, and allergic reactions.

2.4 | Skin ultrasonography

A Dermascan C ultrasound system (Cortex Technology, Denmark) with a modified 20 Mhz ultrasound probe was used to detect the density of tissue and structures in the dermis in the malar area. The sub-epidermal low echogenic band (SLEB) thickness and skin density were calculated.

2.5 | Skin biomechanical evaluation

Skin firmness and elasticity evaluation was performed using a Cutometer® dual MPA 580 with a 2 mm probe utilizing a negative pressure of 450 mBar for 2 s followed by a 2 s release. Measurements were obtained in the malar area and suction height in mm was used as a measure for firmness (R0), while visco-elasticity was measured by recovery distance as a percentage of suction height (R2).

2.6 | Skin barrier function

Trans Epidermal Water Loss (TEWL) was used to measure the skin barrier function. Measurements were assessed by Tewameter® TM 300 (Courage—Khazaka Electronic GmbH, koln, Germany) in the malar area of the face.

2.7 | Skin hydration

By measurement of electrical capacitance, the water content in the stratum corneum can be assessed and used as a measure for skin hydration. Using a Corneometer CM825 probe connected to a cutometer dual MPA 580 (Courage & Khazaka, Germany) measurements were conducted in the malar area of the face. Measurements are obtained in arbitrary units as a reference to a factory standard.

2.8 | Clinical evaluations

Dermatologists or qualified technicians assessed the following parameters: Crow's feet wrinkle clinical scores according to Bazin et al. on a scale from 0 to 6 with 6 being the highest grade of wrinkles, pigmentary spot density score according to Bazin et al. on a scale from 0 to 7 with 7 being the highest grade of pigmentary spot density, smooth aspect and complexion radiance on a scale of 0 to 9 with 9 being the highest.[9]

2.9 | Statistical analysis

Students-*t* or Wilcoxon signed ranks test were performed for all continuous data comparisons between day 0 and day 28 and 56 respectively. A significance level of 95% was adopted ($\alpha = 0.05$). The study was conducted as a before and after study with D0 serving as a baseline, resulting in an evaluation of relative change within each subject.

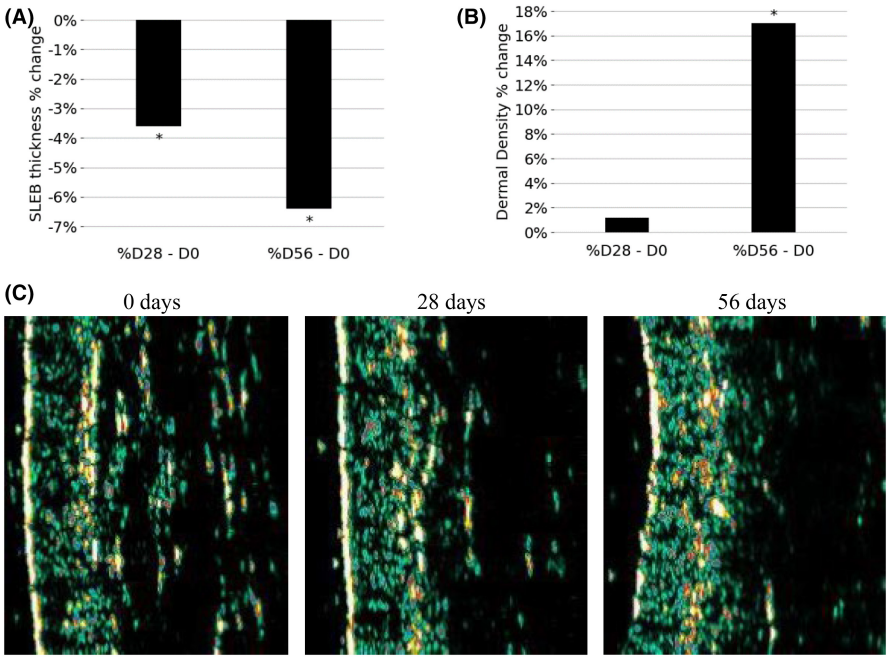

FIGURE 1 Ultrasonographic assessment shows reduced SLEB thickness and increased dermal density (A) Bars showing mean SLEB % change with subjects' day 0 serving as a reference. (B) Bars showing mean Dermal Density % change with subjects' day 0 serving as a reference. (C) Ultrasonography images of Subject #23 after 0 days, 28 days, and 56 days of applying PO. Significance levels in comparison to baseline values were tested by the Wilcoxon signed-rank test for paired data. * indicates $p < 0.05$.

TABLE 2 Anti-aging efficacy ($n = 21$). Values are expressed as the mean of the 21 subjects with ±SD.

	Time point		
Parameters	**D0**	**D28**	**D56**
SLEB thickness (μm)	261 ± 69	245 ± 53*	237 ± 55*
Dermal Density (pixel/mm^2)	325 ± 58	324 ± 87	368 ± 76*
Skin firmness (R0) (μm)	264 ± 38	235 ± 37*	228 ± 37*
Skin elasticity (R2) (%)	57.8 ± 4.5	60.3 ± 4.7*	61.8 ± 4.4*
Skin hydration (AU)	34.1 ± 6.9	40.3 ± 10.3**	51.3 ± 10**
TEWL (g/h/m^2)	9.1 ± 2	9.1 ± 1.4	8.5 ± 1.3**
Crow's feet wrinkles score	3.0 ± 0.8	2.5 ± 0.8*	2.3 ± 0.8*
Spots score	3.5 ± 0.8	2.9 ± 0.8*	2.5 ± 0.8*
Smoothness of the skin score	4.2 ± 0.7	5.0 ± 0.7*	5.5 ± 0.7*
Complexion radiance score	4.1 ± 0.6	5.2 ± 0.6*	5.9 ± 0.5*

*Significance different at $p < 0.05$ in comparison to D0 baseline values by Wilcoxon signed-rank test for paired data. **Significance different at $p < 0.05$ in comparison to D0 baseline values by students t-test.

3 | RESULTS

3.1 | Product compatibility, safety, and acceptability assessment

A total of 23 subjects were enrolled in the trial with 21 completions of treatment and 2 dropouts (Table 1). All subjects tolerated the treatment with PO with no adverse reactions ascribable to the product, emphasizing the safety of topical application of ointment formulation(s) with live *L. plantarum* LB244R. In essence, the product showed very good dermatological compatibility and acceptability.

3.2 | Skin ultrasonography

The mean SLEB thickness was significantly reduced from 261 ± 69 μm to 245 ± 53 μm after 28 days of use (−3.6% reduction, $p < 0.05$) and to 237 ± 55 μm after 56 days (−6.4% reduction, $p < 0.05$). Dermal density was increased from 325 ± 58 pixel/mm^2 to 368 ± 76 pixel/mm^2 after 56 days. Thus, PO had a positive effect on SLEB thickness and dermal density in subjects (Figure 1 and Table 2).

3.3 | Skin biomechanical evaluation

Skin firmness measurements were reduced by 10.8% ($p < 0.05$) after 28 days and 13.1% ($p < 0.05$) after 56 days of application (Table 2). In addition, the skin elasticity (R2) was increased by 4.70% and 7.40% ($p < 0.05$) after 28 and 56 days, respectively (Figure 2 and Table 2).

3.4 | Skin barrier function

TEWL was significantly reduced from 9.1 ± 2.0 g/h/m^2 on day 0 to 8.5 ± 1.3 g/h/m^2 on day 56.

3.5 | Skin hydration

Skin hydration on day 0 was measured to be 34.1 ± 6.9 AU. This was significantly increased after both 28 days (40.3 ± 10.3 AU) and 56 days (51.3 ± 10.0 AU) (Figure 4).

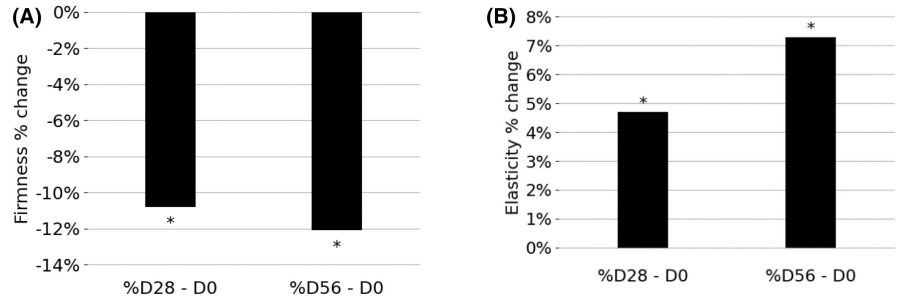

FIGURE 2 Biomechanical properties of the skin measured by a cutometer improved in terms of firmness (R0) and elasticity (R2). (A) Bars showing mean skin firmness % change with subjects' day 0 serving as a reference. (B) Bars showing mean skin elasticity % change with subjects' day 0 serving as a reference. Significance levels in comparison to baseline values were tested by the Wilcoxon signed-rank test for paired data. * indicates $p < 0.05$.

3.6 | Clinical evaluations

The topical application of PO had a positive effect on all clinical aging parameters assessed, including Crow's feet wrinkles, pigmentary spots density, smoothness, and complexion radiance (Figure 3).

The Crow's feet wrinkles were significantly reduced after 28 days (−17.2%; $p < 0.05$) and 56 days (−22.4%; $p < 0.05$) of use. The pigmentary spot density was also significantly reduced after 28 days (−16.3%; $p < 0.05$) and 56 days (−27.5%; $p < 0.05$) of use.

The skin smoothness was significantly increased after 28 days (19.4%; $p < 0.05$) and 56 days (33.5%; $p < 0.05$) of use. The complexion radiance was also significantly increased after 28 days (26.4%; $p < 0.05$) and 56 days (44.4%; $p < 0.05$) of use (Table 2).

4 | DISCUSSION

With the increasing age of the westernized population, treatments that can prevent and rejuvenate signs of skin aging are receiving increased interest due to cosmetic and economic potential. In this clinical study, the anti-aging efficacy and the tolerability of a PO containing live *L. plantarum* LB244R® were evaluated. Anti-aging efficacy on facial skin was evaluated by assessing SLEB thickness, dermal density, skin firmness and elasticity, skin hydration, TEWL, crow's feet wrinkles, spots, smoothness, and complexion radiance and comparing them to baseline measurements.

SLEB has previously been shown to quantify cutaneous senescence, with the thickness expressing the degree of cutaneous aging.[10] Therefore, SLEB has been shown to work well in quantifying aging, especially in photo-exposed areas, such as the malar region. However, due to alternating thicknesses of the different skin layers, SLEB values can vary which can make a comparison to other studies difficult. In this clinical trial, the SLEB thickness was significantly reduced both after 28 and 56 days compared to the baseline (Figure 1 and Table 2).

A study by Crisan et al. examined changes in skin thickness according to age. The study showed that after 40 years of age, SLEB increased, especially in photo-exposed areas.[11] As they assessed different body/skin areas than assessed in this study, values are not directly comparable.

One of the primary aging signs of the skin is the accumulation of cellular senescence, associated with a decreased dermal density.[12] PO showed the ability to increase dermal density significantly after 56 days. This increased dermal density is not only indicative of PO being able to slow tissue degeneration but also of better barrier function.

Ohshima et al. found that the suction distance increases with age.[13] In this study, the suction distance was significantly reduced after 28 and 56 days of application, showing increased firmness. Skin elasticity was elevated after 28 and 56 days of treatment. The recovery distance can be used to find a value for skin elasticity, clearly showing that PO can positively modulate aging skin by increasing elasticity as well as firmness (Figure 2 and Table 2).

When TEWL is increased, hydration is lost, resulting in dull, dry-looking skin, and resulting in impaired barrier function. TEWL is considered an objective measurement of skin (barrier) integrity, and it is known that it is increased with age.[14] The PO was able to significantly reduce the TEWL after 56 days, probably mediated by increasing barrier integrity. Additionally, several studies have found that exposure to pollution increases the TEWL and that this is thought to be caused by radical damage.[14] Radical damage also accumulates with age. LAB have previously been shown to have antioxidant activity, potentially the TEWL decrease could be caused by the alleviation of oxidative stress.[15] In addition to the decreased TEWL, skin hydration was elevated after both 28 and 56 days of treatment (Figure 4). Ingestion of probiotics has also previously shown ability to decrease TEWL in the skin and increase the barrier function.[16] Mode-of-action has been determined as affecting transcriptional regulation and anti-inflammatory properties. Studies have found that ingestion of *Bifidobacterium breve* could alleviate photo-aging.[17] These studies found that *B. breve* could alleviate TEWL, improve skin hydration, inhibit epidermal thickening, reduce IL-1β levels and elastase activity as well as attenuated the damage to the tight junctions after UV-induced irradiation. Another study investigated the ingestion of *L. plantarum* HY7714 after UV-B-induced photo-aging. They found that ingestion could rescue procollagen expression mediated through a matrix metalloproteinase ((MMP)-1).[18]

All clinically assesed parameters were improved concerning aging signs (Figure 3). Crow's feet and spot score were both significantly

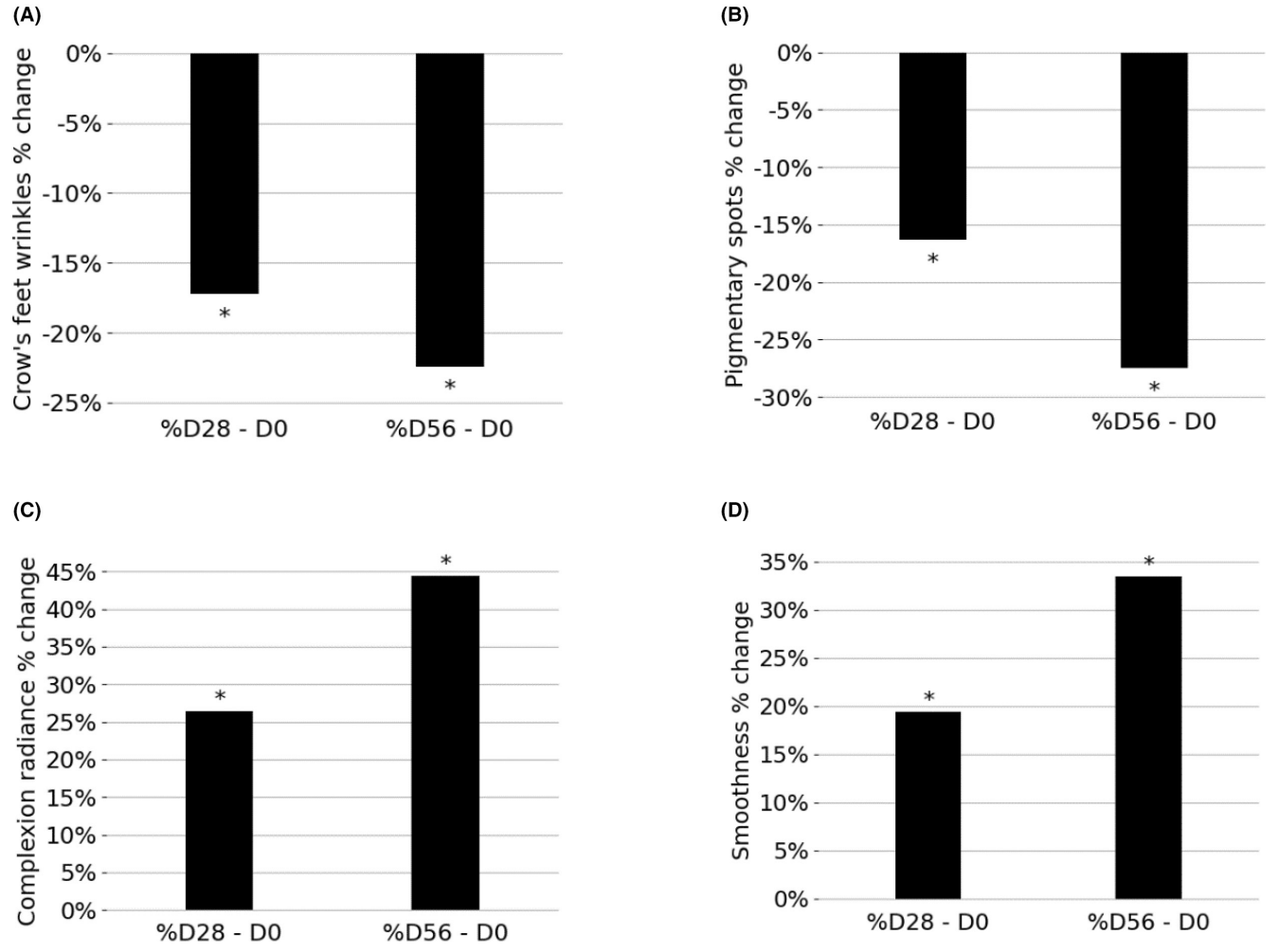

FIGURE 3 Clinical assessment of Crow's feet wrinkles, pigmentary spots density, complexion radiance, and smoothness. (A) Bars showing mean Crow's feet wrinkles % change with subjects' day 0 serving as a reference. (B) Bars showing mean pigmentary spots density % change with subjects' day 0 serving as a reference. (C) Bars showing mean complexion radiance % change with subjects' day 0 serving as a reference. (D) Bars showing mean smoothness % change with subjects' day 0 serving as a reference. Significance levels in comparison to baseline values were tested by the Wilcoxon signed-rank test for paired data. * indicates $p < 0.05$.

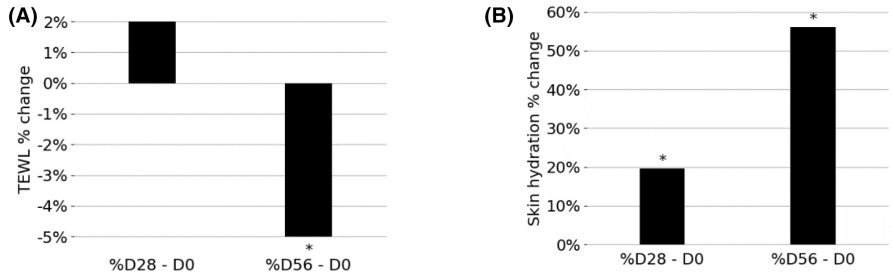

FIGURE 4 Skin barrier function is improved as well as hydration is increased. (A) Bars showing mean TEWL % change with subjects' day 0 serving as a reference. (B) Bars showing mean skin hydration % change with subjects' day 0 serving as a reference. Significance levels in comparison to baseline values were tested by students' *t*-test. * indicates $p < 0.05$.

reduced after both 28 and 56 days of application. Smoothness and complexion radiance of the skin was elevated with statistical significance after both 28 and 56 days of treatment. All of these parameters are based on subjective evaluations, but as it is evaluated by trained professionals, this is no larger concern (dermatologists and trained technicians). Similarly, for self-assessments done through questionnaires, all data trends were the same, with improvement in all aging parameters (data not shown).

The obvious limitations of this study include the lack of a placebo control as well as the small sample size ($n = 21$). This study was a baseline-controlled trial, where before and after treatment were compared to establish anti-aging efficacy. The main limitation is

inferring causality between the treatment and a subsequent change in parameters measured, and it is uncertain whether all of these effects are caused solely by the probiotic strain, as the moisturizing components in the ointment can also have effects. For example, shea butter is known to have anti-aging effects.[19]

Although previously topical application of probiotics has mostly been used to alleviate skin diseases such as acne vulgaris, atopic dermatitis, and psoriasis, there is an increased number of probiotic products being targeted toward aging skin.[20] A study by Kang et al. found that plant extracts fermented with LAB could successfully alleviate UV-induced stress in an in vitro skin model.[21] Mode of action (MoA) was found to be a reduction in elastase activity, increased expression of collagen type I, reduced collagenase activity and increased expression of moisture factors and enzymes with antioxidant activity. Another study by Notay et al. found that topical application of *Nitrosomonas eutropha* could reduce facial wrinkle depth and hyperpigmentation.[6] These studies show similar findings to that of this article, with aging parameters improving after the topical application of probiotics as well as their fermented products. At present, most studies have investigated the oral ingestion of probiotics, and studies working with topical application remain sparse.[22] Previously, it has been found that oral ingestion can alleviate dermal inflammatory cells and increase complexion radiance.[23]

It is possible that microbiome modulation may affect aging parameters. One recent study found that the amount of *Cutibacterium* spp. and *Lactobacillus* spp. decreased with age.[3] They suspected that it was age-related changes in physiology that resulted in subsequent microbiome change, but perhaps the microbiome may be able to modulate host physiology, thereby providing a more hospitable environment for them, allowing for colonization. Li et al. also found that microbiomes play a regulatory role in aging, further reaffirming that microbiome modulation may affect aging.[24]

Previous studies have shown that oral intake of probiotics can affect the skin, indicating that the effects are systemic.[5] A randomized double-blind, placebo-controlled study by Lee et al. found that oral intake of *Lactobacillus plantarum* HY7714 improved skin hydration, reduced wrinkles, increased skin "gloss" and skin elasticity.[25]

Studies in animal models and human clinical trials have shown that probiotic products can slow intrinsic and extrinsic aging. This is thought to work by restoring the acidic pH of the skin, alleviating oxidative stress, attenuating, and even restoring photoaged skin, improving the barrier function of skin, and enhancing hair quality.[5]

The factors responsible for the improvement seen in all skin-aging parameters following the application of the PO are likely due to all factors mentioned above, as well as their intricate interactions. To the best of our knowledge, this study is the first clinical study using live probiotic LAB topically in an ointment formulation to mitigate aging signs. Moreover, there was an improvement in all measured parameters, which clearly shows the immense potential of LAB in topical use for aging skin.

In essence, knowledge is lacking concerning the anti-aging MoA caused by the topical application of LAB. Additionally, the economic potential is clearly seen in the amount of filed patents. For future studies, it is important to figure out the specific MoA. Whether the anti-aging efficacy is mediated through reduction of senescence, specific mammalian signaling, immune modulation, and inhibition of inflammation, by microbiome modulation, or by directly affecting the ECM and collagen/elastan, will be for future studies to uncover.

5 | CONCLUSION

Despite the limitations of this study, PO significantly improved all measured parameters with statistical significance after 56 days of treatment, clearly showing the potential of PO as an anti-aging cosmetic product. To the best of our knowledge, this is the first study investigating the topical application of LAB in a clinical trial, and MoA behind the anti-aging efficacy cannot be determined from this study. Future studies need to delve further into this. Additionally, larger studies with placebo-control and follow-up periods could be valuable in confirming the findings from this study.

AUTHOR CONTRIBUTIONS

The authors confirm their contribution to the paper as follows: Study conception and design: Helena Falholt Elvebakken, Anders Bech Bruntse, Søren Kjærulff. Data collection: PhD trials. Analysis and interpretation of results: Helena Falholt Elvebakken, Anders Bech Bruntse. Draft manuscript preparation: Helena Falholt Elvebakken, Charlotte Vedel, Søren Kjærulff, All authors reviewed the results and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

Helena Falholt Elvebakken, Anders Bech Bruntse, Charlotte Vedel, and Søren Kjærulff are employed at Lactobio A/S at the time of writing this manuscript. Lactobio A/S is the manufacturer of the investigated probiotic oil-based ointment formulation. The study was carried out by an independent entity, Ph.D.-Trials (Portugal) financed by Lactobio.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENTS

We confirm that all methods were carried out in accordance with relevant regulations and guidelines. The study was conducted in spirit of Good Clinical Practice Guidelines and general principles of Law 46/2004 of August 19th. The protocol and test conditions were reviewed by the internal review board (Opinion No. 8397/2022) and the standard protocol was submitted to the ethical commission of PhD Trials (From January 4th, 2021).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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