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# Biological Effects of a *Bacillus sp*. Ferment Extract through the Protection of Key Features Associated with Aging in Human Skin Biopsies

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## Abstract

Aging is an inevitable and multifaceted biological process that significantly impacts human skin at various levels, leading to structural and functional changes. These changes include a decline in epidermal stem cell functionality, alterations in the dermal-epidermal junction (DEJ), and the deterioration of the extracellular matrix (ECM). Telocytes, a type of interstitial cell found in the dermis, play a crucial role in maintaining skin health. They are also suggested to be involved in the organization and maintenance of the ECM, and their role in skin aging is currently under investigation. In this study, we used a comprehensive age-induced skin model developed by exposing human skin biopsies to both ultraviolet (UV) irradiation and glycation stress, creating a suitable tool to recapitulate the skin aging features caused by extrinsic and intrinsic factors. Changes in the ECM density and in the organization and integrity at the collagen network level were illustrated using this *ex vivo* model. Moreover, we validated that the aging process can be delayed by treatment with a newly developed *Bacillus sp.* ferment extract. The extract improved DEJ integrity and supported the epidermal stem cell niche in skin biopsies from an elderly donor. Furthermore, the protection of ECM features associated with aging conditions after *Bacillus sp.* treatment was also confirmed. Finally, we demonstrated that the new *Bacillus sp.* ferment extract increased the number of telocytes in human skin biopsies from an elderly donor and enhanced the proliferation rate of isolated telocytes in culture, providing a hypothetical explanation for its anti-aging mechanism of action.

## Keywords

Anti-Aging, Epidermal Stemness, Dermal-Epidermal Junction, Extracellular

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Matrix, Telocytes

## 1. Introduction

Aging is a complex biological process that affects all layers of human skin, resulting in notable structural and functional alterations [1] [2]. The decline in the functionality and regenerative capacity of epidermal stem cells is one of the key aspects of skin aging. Epidermal stemness diminishes, resulting in a reduced ability to repair and regenerate the skin [3] [4], contributing to the thinning of the epidermis, and substantially increasing the vulnerability of older individuals to skin damage, compromised barrier function, and reduced skin hydration [2]. Among others, Cytokeratin 15 (CK15) protein has been proposed to play a key role in maintaining basal epidermal cell stemness [5].

Skin aging also has a deleterious impact on the dermal-epidermal junction (DEJ), the structure that connects the epidermal and dermal layers of the skin [6]. As aging progresses, the DEJ undergoes structural changes, including a decrease in its undulating morphology and a reduction in the number of essential components, such as hemidesmosomes and anchoring fibrils. Laminin 5, an essential adhesion protein within the DEJ structure, has been described to be reduced in aged skin [7] [8]. These alterations compromise the mechanical stability of the skin, impairing the balance between cell renewal and proliferation within the epidermis and further exacerbating the visible signs of aging [7].

The extracellular matrix (ECM) is significantly impacted by the skin aging process. Mainly composed of proteins such as collagen and elastin synthesized by dermal fibroblasts, the ECM provides structural support to the skin and confers essential mechanical properties such as stiffness and elasticity [2]. With increasing age, the number of fibroblasts declines, leading to a reduction in the synthesis of collagen and elastin [1]. Extrinsic and intrinsic factors contributing to the skin aging process also induce ECM degradation and disorganization, resulting in altered mechanical properties [1]. Previous research has indicated that the reticular dermis of aged skin exhibits a reduced dispersion of collagen directionality, resulting in a more parallel orientation to the superficial epidermal axis compared to younger skin [9]. Moreover, the integrity of the collagen fibers deteriorates during skin aging, further compromising the structural and functional properties of the skin. These changes collectively manifest as a loss of skin firmness and elasticity and contribute to the formation of fine lines and wrinkles, making the skin appear aged [1] [2].

Telocytes are crucial interstitial cells in maintaining skin health, found in the dermis and associated with connective tissue elements such as blood vessels and nerve endings [10]. Characterized by their unique morphology, with long and thin extensions called telopodes, telocytes facilitate communication with other cells, including fibroblasts, immune cells, microvascular endothelial cells, and stem cells [10]-[13].

They contribute to supporting epidermal stem cells by promoting their proliferation and differentiation, essential for skin regeneration [10] [14]. Additionally, telocytes are thought to help control and organize the ECM, maintaining tissue homeostasis [15]-[17]. It has been suggested that their impairment could lead to the disorganization of collagen and elastic fibers, potentially contributing to aging-related changes in the ECM of the skin [14] [18] [19]. The relationship between telocytes and aging is undergoing research, with some studies suggesting that they might contribute to tissue-associated aging features [14] [20]. Protein expression profiling of human lung telocytes showed that telocytes might inhibit oxidative stress and cellular aging [21]. In the human heart, telocytes seem to provide support to cardiac stem cells, and their number significantly decreases in adults compared to younger groups, which may contribute to the heart aging process [22].

Understanding the underlying molecular mechanisms of skin aging and finding ways to reverse it are crucial for promoting healthy aging and improving overall cellular health [23]-[25]. Topically applied fermented skincare ingredients have gained increasing attention due to their high concentration of various skin-nourishing nutrients and bioactive components and their low potential for skin irritation. *Bacillus sp.* ferment extract has been shown to provide potent skin benefits *in vitro* and protects skin from aging [26] [27].

In this study, to evaluate the effect of aging on the ECM, a comprehensive age-induced skin model was developed by exposing human skin biopsies to both extrinsic factors, such as ultraviolet (UV) irradiation, and intrinsic factors, such as glycation. These factors are significant contributors to skin aging [28] [29] and using both in combination provides a more realistic approach to reproducing the features of aged skin. Using this model, we characterized the structural and organizational changes that occur in the ECM during the skin aging process. The reduction of ECM density, changes in collagen directionality, and deterioration of collagen fiber integrity were demonstrated under aged-induced conditions in human skin biopsies. We also validated that the aging process can be delayed by treatment with a new *Bacillus sp.* ferment extract by promoting DEJ integrity and supporting the epidermal stem cell niche in human skin biopsies from aged donors. Additionally, the protection of the ECM features was demonstrated after the treatment with the ferment extract in the age-induced skin model. Finally, the new *Bacillus sp.* ferment extract was shown to promote the number of telocytes in aged human skin biopsies, as well as to induce the proliferation of isolated telocytes in culture, suggesting a plausible explanation for its anti-aging mechanism of action.

## 2. Materials and Methods

### 2.1. Obtention of the Bacterial Ferment Extract

*Bacillus sp.* ferment extract (Telophi™ biotech ingredient) was provided by Lipotec S.A.U., a subsidiary of Lubrizol. The extract was developed from a marine strain of *Bacillus sp.* isolated from a sponge colony inhabiting the Florida Keys area (USA). The biotechnological process involved the fermentation of the bacteria in stirred tank bioreactors, followed by extraction and clarification to remove biomass.

## 2.2. Aged Human Skin Biopsies Culture

Human skin biopsies from abdominal plastic surgery from a 68-year-old healthy woman were used to evaluate DEJ integrity (Laminin 5), epidermal stemness potential (CK15), and the number of telocytes. The ferment extract was applied to day 0 (D0), D2, D5, and D7, at 250 µg/mL. Control skin biopsies did not receive any treatment except for the renewal of culture medium (untreated control). Three skin samples for each treatment condition were cultured and analyzed after 8 days of treatment.

## 2.3. Induction of Aging Conditions in Human Skin Biopsies

Human skin biopsies from abdominal plastic surgery of a 51-year-old healthy woman were used. To evaluate the ECM features, aging conditions were induced by exposing skin samples to UV irradiation in combination with glycation stress. The skin biopsies were irradiated at a dose of 9 $J/cm^2$ of UVA using a UV simulator (Vibert Lourmat RMX 3W) on D0, D2, D4, D6, and D8. Glycation stress was induced by treating the skin biopsies with 500 µM methylglyoxal (MG, Sigma) on D4, D6, and D8. *Bacillus sp.* ferment extract was applied to D0, D2, D4, D6, and D8 at 250 µg/mL. Two types of control biopsies were used: the "untreated control" (biopsies neither subjected to aging induction nor treated with the ferment extract) and the "aging-induced control" (biopsies subjected to aging-induced conditions without ferment extract treatment). Three skin samples for each treatment condition were cultured and analyzed after 10 days of treatment.

## 2.4. Histological Processing

After fixation for 24 hours in buffered formalin, the skin samples were dehydrated using a Leica PEARL dehydration automat and impregnated in paraffin using a Leica EG 1160 embedding station. Skin sections were made using a Leica RM 2125 Minot-type microtome, and the sections were mounted on Superfrost® histological glass slides.

## 2.5. Histological Staining

Laminin 5 immunostaining was performed with an anti-laminin 5 antibody (Santa Cruz). CK15 immunostaining was performed with an anti-CK15 antibody (Santa Cruz). CD34/PDGFRα co-immunostaining was performed on skin sections with an anti-CD34 antibody (Santa Cruz) and an anti-PDGFRα antibody (Thermo Scientific). The nuclei were post-stained using propidium iodide. Elastin and collagen type I immunostaining were performed with an anti-elastin antibody (Novotec) or an anti-collagen type I antibody (Abcam). The nuclei were counterstained with propidium iodide. Total collagen staining was performed with a Red Sirius F3B solution (Picro-Sirius).

## 2.6. Image Analysis Method and Statistical Analysis

For each treatment condition, 9 images were analyzed. For each image, the percentage of the region of interest covered by the specific staining was determined by image analysis using the CellSens software. Statistical analysis was performed using the Student's t-test for independent samples.

## 2.7. Collagen Directionality Analysis

Picro-Sirius red stained images were used to infer the preferred orientation of the collagen network as previously reported [30] [31] and using the "Directionality" method on ImageJ software. The method computes a histogram that shows the amount of collagen structures in each direction. The preferred direction represents the center of the Gaussian distribution, and the collagen directionality dispersion corresponds to the standard deviation of the preferred collagen directionality and is represented by the Gaussian curve width.

## 2.8. Analysis of Collagen Fiber Integrity by Polarization Analysis

Collagen fiber integrity was quantified using the XPolar® technology (Kmax Innovative System, France) on 51-year-old skin biopsy sections. This advanced approach allows for precise measurement of collagen bundle birefringence with submicrometric resolution, providing detailed insights into changes in collagen polarization. The polarization changes are represented by a dimensionless number called Kmax [32]. The Kws parameter (averaged Kmax), which is directly related to collagen fiber integrity, was calculated for each condition. A higher Kws value correlates with improved collagen fiber integrity.

## 2.9. Isolation and Characterization of Telocytes from Human Skin Biopsies

Human skin samples from abdominal and breast plastic surgeries of 37- and 44-year-old healthy women were dermatomized and minced into millimetre-sized pieces. After enzymatic digestion, the cell suspension was filtered through a 40 μm cell strainer, centrifuged, and the pellet was resuspended in culture medium. The cells were seeded in sterile culture flasks and incubated at 37˚C in 5% $CO_2$ humidified air for 2 hours to allow fibroblast attachment. The unattached cells, containing telocytes, were reseeded into new culture flasks, and the medium was replaced every 2 days thereafter [33]. To eliminate fibroblasts from the culture, the first medium changes were performed by adding FibrOut™ System 6 (CHI Scientific) to the culture medium. The identification of telocytes in culture was performed by a triple immunocytochemistry by incubating telocytes with a mixture of primary antibodies: anti-CD34 antibody (R&D Systems), anti-PDGFRα antibody (Life Technologies), and chicken anti-Vimentin antibody (Abcam), as previously described [10] [11]. Next, cells were incubated with a mixture of secondary antibodies and Hoechst (Life Technologies) for nuclei visualization. Microscopical observations were performed with a high-content screening system (Operetta®

High-Content Imaging System, PerkinElmer, Inc.).

### 2.10. Telocytes Proliferation Assay

Isolated telocytes were seeded in 96-well plates in culture medium and treated with *Bacillus sp.* ferment extract at 50 µg/mL. Cells treated with medium alone were used as the basal control. After 24 hours of incubation at 37˚C in a humidified, 5% $CO_2$ atmosphere, cells were fixed, and double immunocytochemistry was performed with CD34 and PDGFRα antibodies, as previously described. The number of double positive cells, corresponding to telocytes, was quantified with the Harmony® Analysis Software (PerkinElmer, Inc.).

## 3. Results

### 3.1. Quantification of Laminin 5 and Cytokeratin 15 in Aged Human Skin Biopsies

Laminin 5, a key structural component involved in the integrity of the DEJ, was studied in skin biopsies of an elderly donor. Previous studies have reported that Laminin 5 protein levels diminish with aging, potentially impairing epidermal differentiation and reducing cohesion between the dermal and epidermal layers [7] [8]. Consistent with these findings, **Figures 1(A)-(B)** depict moderate Laminin 5 staining at the DEJ in aged skin biopsies, along with a flattened morphology. Treatment of aged skin biopsies with a *Bacillus sp*. ferment extract significantly enhanced Laminin 5 levels by 29.9% compared to the untreated condition. This enhancement is visualized as a more pronounced green fluorescence signal at the DEJ after treatment. Moreover, rete ridges along the DEJ exhibited a more undulating morphology post-treatment, suggesting a rejuvenation effect [7].

CK15, a marker associated with the stemness potential of the basal layer of the epidermis [5], was also examined in skin biopsies from the same elderly donor. **Figures 1(C)-(D)** show low detection of CK15 in the basal layer of the epidermis under untreated conditions, confirming reduced proliferative potential of epidermal basal cells. Treatment with *Bacillus sp.* ferment extract led to a statistically significant increase of 276.0% in CK15 protein levels versus the untreated condition, as evidenced by enhanced violet staining.

These results suggest that the ferment extract not only promotes the DEJ integrity by upregulating Laminin 5 protein levels in aged skin but also supports the population of epidermal basal stem cells, thereby improving epidermal stemness potential in aged human skin biopsies.

### 3.2. Evaluation of Extracellular Matrix Density under Aged-Induced Conditions

Human skin biopsies were subjected to aging-induced conditions through both UV exposure and glycation stress, as detailed in Materials and Methods section. To evaluate the impact of aging on the ECM, immunostaining for elastin and collagen type I, along with total collagen staining, was performed.

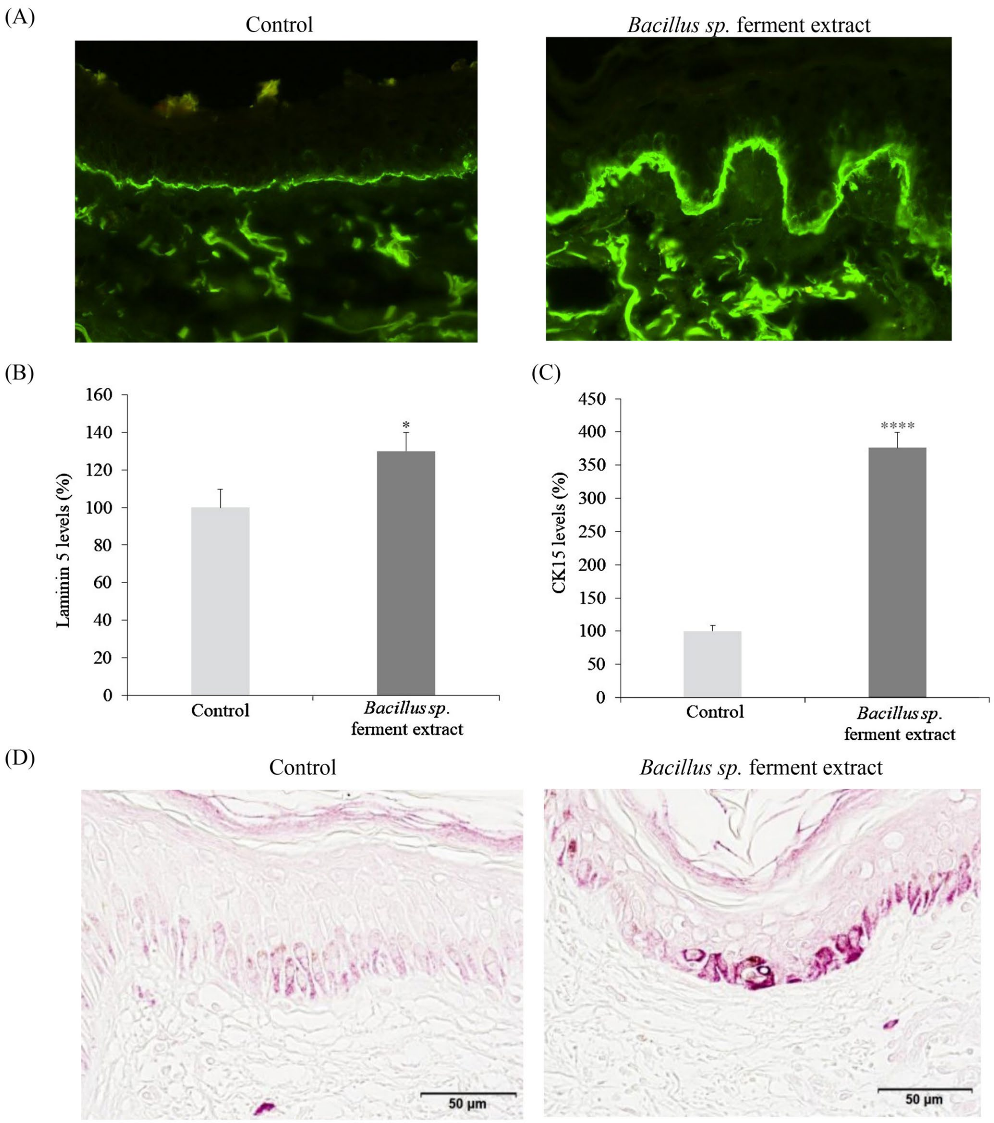


**Figure 1.** Quantification of Laminin 5 in the DEJ, and CK15 in the basal layer of epidermal stem cells niches in elder human skin explants. (A) Representative images of Laminin 5 (green). (B) Quantification of immunofluorescence signals for Laminin 5 levels (%). Statistical significance: $*p < 0.05$, calculated using an unpaired Student's t-test. (C) Quantification of immunofluorescence signals for CK15 levels (%). Statistical significance: $****p < 0.0001$, calculated using an unpaired Student's t-test. (D) Representative images of CK15 (violet).

Figures 2(A)-(B) show a decrease in elastin levels under aging-induced conditions compared to the untreated control. Moreover, a notable reduction in collagen type I and total collagen levels was observed in the dermis under aging-induced conditions compared to the untreated control (Figures 2(C)-(D), Figures 3(A)-(B)). These results confirm that the aging-induced model in human skin biopsies is suitable for evaluating ECM deterioration during skin aging. Treatment with *Bacillus sp.* ferment extract under aging-induced conditions showed a statistically significant improvement in elastin levels by 97.1% compared to the aging-induced control, demonstrating that the ferment extract helps to maintain elastin density (Figures 2(A)-(B)). Furthermore, the treatment with *Bacillus sp.* ferment extract significantly maintained collagen density, resulting in a statistically significant increase in collagen type I levels of 33.9% compared to the aging-induced control (Figures 2(C)-(D)). Finally, total collagen levels in the dermis were also preserved, as evidenced by denser red staining post-treatment (Figure 3(A)), showing a statistically significant increase of 12.0% compared to the aging-induced control (Figure 3(B)), reaching levels similar to those in the untreated control condition (Figures 3(A)-(B)).

Collectively, these findings demonstrated that *Bacillus sp.* ferment extract safeguards elastin, collagen type I, and total collagen levels under aging-induced conditions, thereby protecting ECM density during skin aging.

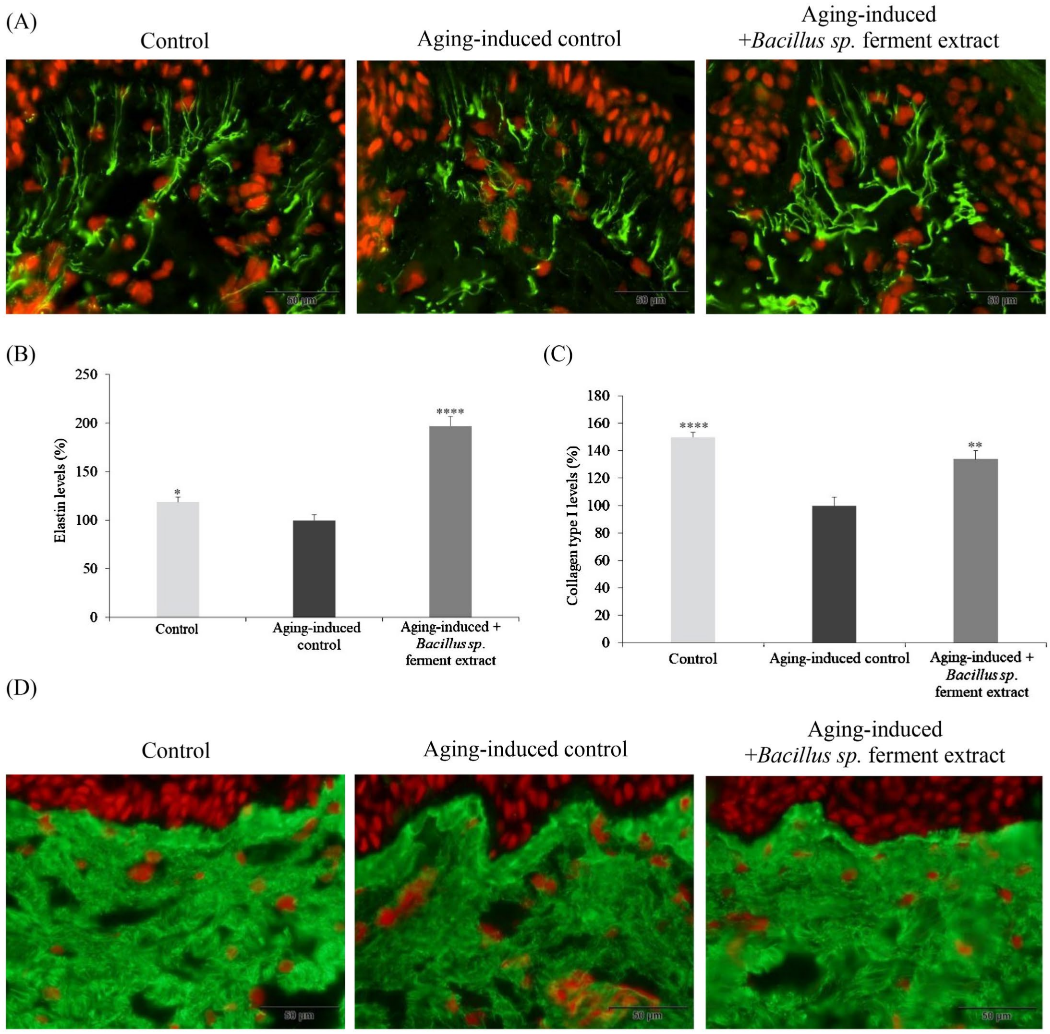


**Figure 2.** Quantification of elastin and collagen type I levels of human skin explants under aging-induced conditions. (A) Representative images of elastin stained in green and cell nuclei in red. (B) Quantification of immunofluorescence signals for elastin levels (%). Statistical significance: *$p < 0.05$, ****$p < 0.0001$, calculated using an unpaired Student's t-test. (C) Quantification of immunofluorescence signals for collagen type I levels (%). Statistical significance: **$p < 0.01$, ****$p < 0.0001$, calculated using an unpaired Student's t-test. (D) Representative images of collagen type I stained in green and cell nuclei in red.

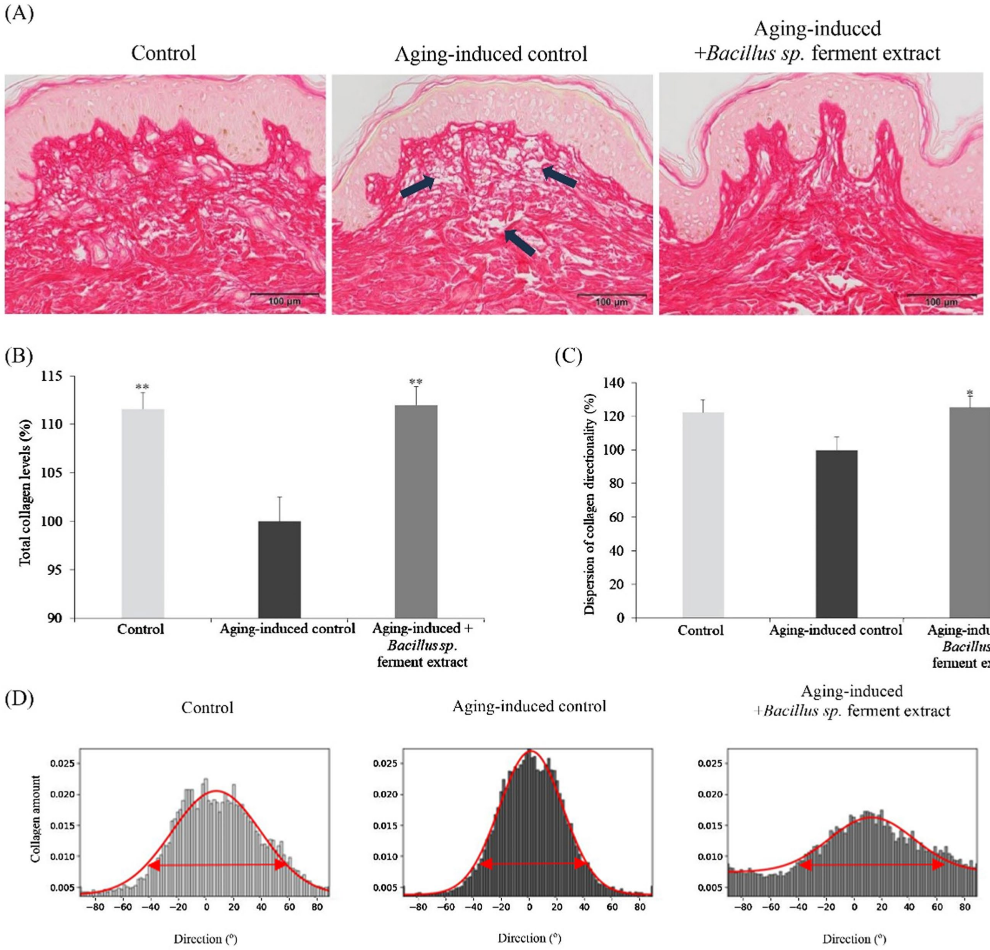


**Figure 3.** Analysis of total collagen expression in human skin explants in the papillary and upper reticular dermis under aging-induced conditions and evaluation of total collagen directionality in the middle reticular dermis. (A) Representative images of total collagen levels (Picro-Sirius red staining). (B) Quantification of total collagen levels (%). Statistical significance: $**p < 0.01$, calculated using an unpaired Student's t-test. (C) Dispersion of collagen directionality (%). Statistical significance: $*p < 0.05$, calculated using an unpaired Student's t-test. (D) Collagen directional dispersion histogram with Gaussian fitting. The standard deviation of the preferred collagen directionality is indicated by the width of the Gaussian curve (red arrow).

### 3.3. Measurement of the Collagen Organization under Aging-Induced Conditions

The dispersion of collagen directionality, a measure of collagen network organization, was also examined in this study under aging-induced conditions. As previously described, reduced collagen directionality promotes a parallel orientation of collagen fibers during aging, diminishing the skin's mechanical properties [9]. Consistent with this, a reduction in collagen directionality dispersion was observed under aging-induced conditions (**Figures 3(C)-(D)**). Treatment with *Bacillus sp.* ferment extract significantly enhanced collagen directionality dispersion by 25.3% compared to the aging-induced control (**Figure 3(C)**). This finding indicates that the ferment extract helps protect against collagen network disorganization during skin aging, a key factor in dermal layer deterioration [1].

### 3.4. Collagen Fiber Integrity Quantification under Aging-Induced Conditions

Collagen fiber integrity was evaluated by measuring collagen bundle birefringence, as described in the Materials and Methods section, and X-Polar® images were obtained (Figure 4(A)). Aging-induced conditions resulted in a reduction in the Kws parameter, indicating decreased collagen fiber integrity. Treatment with *Bacillus sp.* ferment extract significantly increased collagen fiber integrity by 44.5% in a statistically significant manner compared to the aging-induced control (Figures 4(A)-(B)), reaching levels similar to the untreated control. This result demonstrates the protective effect of the ferment extract against collagen fiber deterioration during skin aging.

Overall, our results indicate that *Bacillus sp.* ferment extract not only protects skin elastin and collagen type I levels but also helps to maintain ECM organization during skin aging, thereby preserving collagen fiber integrity.

### 3.5. Determination of Telocytes Number in Aged Human Skin Biopsies

Co-immunostaining for CD34 and PDGFRα was performed to detect telocytes in skin biopsies from an elderly donor. Figure 5(A) shows minimal telocyte content

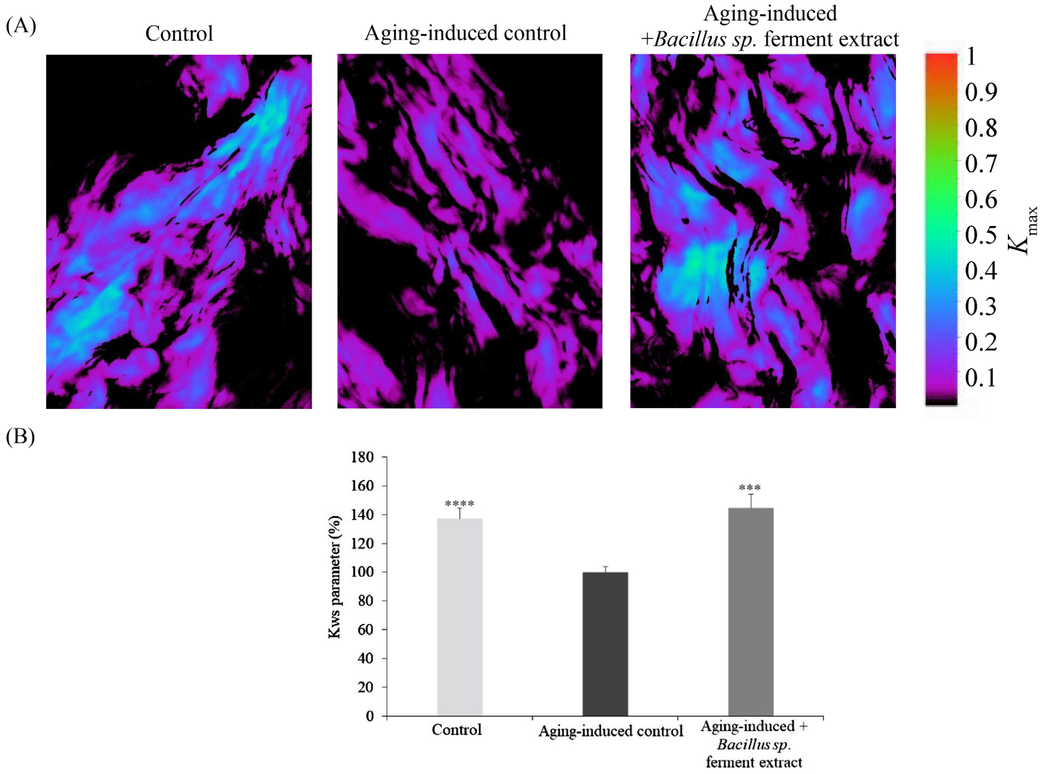


**Figure 4.** Collagen fiber integrity in human skin explants under aging-induced conditions on the middle reticular dermis. (A) Representative X-Polar® images. Color scale based on the Kmax value, with darker purple color indicating lower collagen fiber integrity, and vice versa. (B) Kws parameter (%). Statistical significance: ***p < 0.001, ****p < 0.0001, calculated using an unpaired Student's t-test.

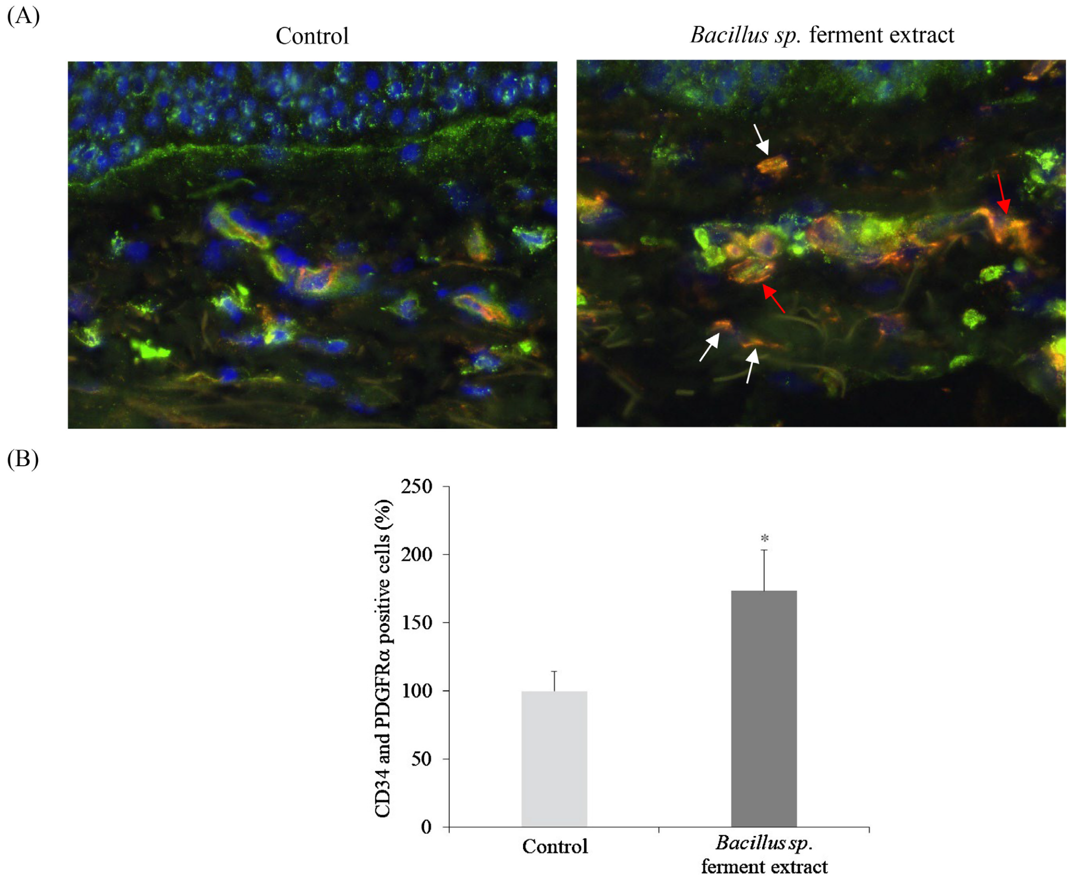


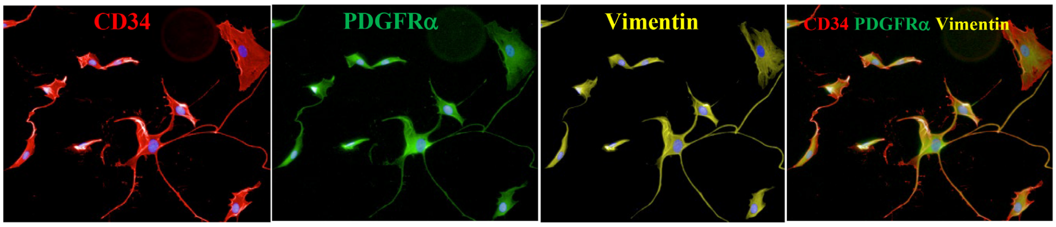
**Figure 5.** Quantification of telocytes in elder human skin explants. (A) Representative images of CD34/PDGFRα co-immunostaining. CD34 was detected in red fluorescence and PDGFRα in green. The co-immunostaining signal, which identifies telocytes, is visualized in yellow-orange and cell nuclei in blue. White arrows indicate telocytes in the interstitial space and red arrows telocytes around microvascular vessels. (B) Quantification of co-immunofluorescence signals for CD34/PDGFRα (%). Statistical significance: $^*p < 0.05$, calculated using an unpaired Student's t-test.

under untreated conditions, with few cells observed along the dermis, primarily around microvascular vessels. Treatment with *Bacillus sp.* ferment extract significantly increased the number of telocytes in the dermis, as shown in **Figure 5(A)**. This enhancement was more prominently distributed throughout the interstitial space of the dermal compartment and around microvascular vessels. Quantitative analysis (**Figure 5(B)**) revealed a statistically significant increase of 73.5% in co-staining signal compared to the control condition, demonstrating that the ferment extract can increase telocytes number in aged skin biopsies.

### 3.6. Isolation and Proliferation Assay of Cultured Telocytes

Isolated telocytes from human skin biopsies were maintained in culture and characterized by fluorescence immunostaining. **Figure 6(A)** shows cells triple positive for CD34, PDGFRα, and Vimentin, confirming their identity as telocytes. Moreover, these cells exhibited the characteristic morphology of telocytes, featuring a small cell body and long moniliform telopodes. Connections between cells were also observed in the established cultures through telopodes [10] [11] [33]. As shown in **Figure 6(B)**, treatment with *Bacillus sp.* ferment extract significantly increased the number of telocytes in the culture by 19.6% compared to the untreated condition.

(A)



(B)

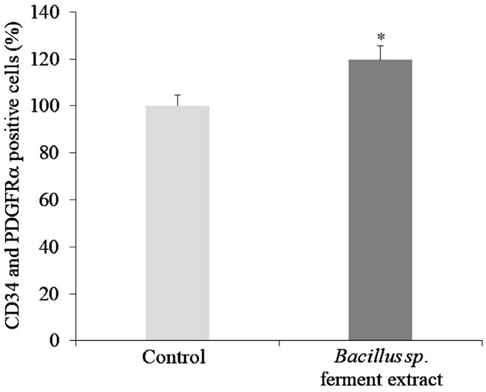


**Figure 6.** Telocytes characterization and quantification in primary cell culture. (A) Representative images of triple immunofluorescent staining for CD34/PDGFRα/vimentin, which identifies telocytes. Cell nuclei in blue. (B) Proliferation assay: quantification of co-immunofluorescence signals for CD34/PDGFRα (%). Statistical significance: $^*p < 0.05$, calculated using an unpaired Student's t-test.

## 4. Discussion and Conclusion

Aging imposes significant structural and functional changes on human skin, including a decline in epidermal stem cell functionality, alterations in the DEJ structure, and the deterioration of the dermal ECM network [1]-[8]. The study of these deleterious features of skin aging is a continuously evolving research field, as scientists strive to elucidate the comprehensive mechanisms underlying these changes. Although there is a wide array of anti-aging compounds currently available for cosmetic and dermatological applications, ongoing advanced research in the anti-aging field continues to pave the way for more efficient and innovative solutions.

Different *in vitro* models have been utilized in the literature to describe the impact of aging and senescence using human skin cells maintained in culture [34]. However, *ex vivo* models based on human skin biopsies provide a more suitable and realistic approach to better understand the detrimental changes that aging imposes on the skin [35]. In alignment with this, several approaches have been developed to reproduce features of skin aging in human biopsies by exposing them to known detrimental factors, such as UV radiation, environmental pollutants, glycation, or by inducing senescence [36]. It is important to note that inducing the aging process in human skin explants has certain limitations. Aging is a multifaceted process involving genetic, environmental, and lifestyle factors that are difficult to replicate accurately *in vitro*. Additionally, human skin aging is influenced by systemic factors such as hormonal changes and immune system interactions, which are not present in isolated skin biopsies [36] [37]. Despite these limitations, in this study, we describe a comprehensive protocol for the induction of aging in human skin biopsies using a combination of UV exposure and glycation stress simultaneously. UV radiation has been shown to trigger the destruction of pre-existing collagen content in the skin, primarily mediated by the activation of matrix metalloproteases (MMPs), whereas glycation promotes the cross-linking of collagen fibers, worsening the structural organization and integrity of the ECM [38] [39]. Therefore, the aging-induced protocol developed using human skin biopsies offers a realistic approach by including both extrinsic (UV) and intrinsic (glycation) factors to reproduce the features of aged skin.

First, we demonstrated the reduction of elastin, collagen type I, and total collagen density in the dermal layer of the skin under aging-induced conditions. We also developed a new *Bacillus sp.* ferment extract capable of protecting against ECM loss during aging induction, thus offering a promising extract intended to preserve ECM density in older skin. Our study also assesses collagen disorganization and the reduction of collagen fiber integrity that occurs with skin aging. Our findings confirm that the dispersion of collagen directionality diminishes under aging-induced conditions, as previously suggested in the literature [9]. Furthermore, we demonstrated by assessing collagen bundle birefringence that collagen fiber integrity is reduced in aging-induced conditions in human skin biopsies. Treatment with the ferment extract helped to protect against changes in collagen directionality and collagen fiber integrity. Therefore, *Bacillus sp.* ferment extract showed to be capable not only of protecting ECM density, but also of preserving ECM organization and collagen integrity from the changes that take place during skin aging. Further research into the organizational and integrity changes of the ECM that occur during the skin aging process can contribute to a better understanding of the reduced mechanical properties described in aged skin [1] [2].

Epidermal stemness potential and integrity of DEJ have also been reported to be reduced in skin samples of older donors compared to younger counterparts [2] [3] [7]. Consistent with these findings, thinning of the epidermis, compromised barrier function, and decreased hydration have been demonstrated in aged skin [2]. Moreover, as aging progresses, the DEJ undergoes structural changes, including a decrease in the number of essential components of this structure, which impacts epidermal-dermal cohesion [7]. Using human skin biopsies from an older donor, our study demonstrated reduced levels of Laminin 5 and CK15 proteins, confirming compromised DEJ integrity and reduced basal stemness potential, respectively. Interestingly, treatment with the *Bacillus sp.* ferment extract was able to increase the levels of Lamin 5 and CK15 in aged skin biopsies, further confirming its anti-aging potential.

Finally, with the aim of delving deeper into the mechanisms underlying the anti-aging efficacy of this ferment extract, we focused on the role that skin telocytes could play. Previous studies have suggested that telocytes could participate in dermal communication, maintenance of epidermal stem cells, and ECM homeostasis [10]-[19]. Additionally, they have been hypothesized to play a protective role in the tissue aging process [14] [20]-[22]. Aligned with this information, our study confirmed a reduced number of telocytes in aged skin tissues, primarily localized around superficial microvessels, as expected [11] [14] [33]. We first demonstrated that the number of telocytes could be increased in human skin biopsies from an older donor by treatment with the *Bacillus sp.* ferment extract. Furthermore, we successfully isolated and characterized skin telocytes from human skin biopsies. The *Bacillus sp.* ferment extract additionally proved to increase the proliferation of isolated telocytes in *in vitro* culture. Based on these results, we suggest that the skin anti-aging benefits achieved by the *Bacillus sp.* ferment extract treatment might be mediated by the positive modulation of skin telocytes. In that sense, our study contributes to providing novel insights into how telocytes content could be regulated in aged skin and opens new possibilities for their modulation as a potential new strategy to minimize the loss of skin functionality that occurs with aging. Nevertheless, further research on this topic is needed to clarify a direct relationship between a reduced number of telocytes and the detrimental skin aging changes observed in our studies.

Collectively, our study describes the development of a comprehensive age-induced skin model using human skin biopsies, which illustrates the deleterious changes in ECM density, organization, and integrity associated with aged skin. Moreover, a new *Bacillus sp.* ferment extract with demonstrated skin anti-aging potential has been developed. Finally, we suggest skin telocytes modulation as a potential mechanism that might mediate this effect. Consequently, the new *Bacillus sp.* ferment extract could be considered a promising new active ingredient to be included in cosmetic or dermatological formulations for rejuvenation purposes.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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