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Lysates of *Bifidobacterium longum* subsp. *iuvenis* alleviate SLS-simulated skin barrier damage in human skin tissue

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1. Introduction

The skin barrier, composed of the stratum corneum, sebum layer, and tight junctions, is the body's first defense against pathogens, pollutants, and UV radiation [1]. It maintains skin homeostasis by preventing excessive water loss [2]. When damaged, it causes increased transepidermal water loss (TEWL), leading to inflammation, infections, and skin sensitivity [3]. Skin barrier repair mechanisms include promoting keratinocyte growth, reconstructing the sebum membrane, reducing inflammation, and antioxidant effects [3-4]. Recently, the skin microbiome's role in strengthening the skin barrier has drawn much attention [5].

Probiotics, live microorganisms beneficial to health in adequate amounts, have long been linked to gut health regulation. New evidence shows their benefits also apply to skin [6]. For example, Sphingomonas hydrophobicum extracts inhibit β-galactosidase, suppress p21 and p16, delaying skin aging [7]. Bifidobacterium adolescentis culture filtrate modulates tyrosinase via antioxidants, reducing melanin and lightening skin. Bifidobacterium longum lysate alleviates skin sensitivity by reducing neuronal reactivity [8]. Moreover, the lysate of Bifidobacterium longum has been demonstrated to alleviate skin sensitivity by dampening neuronal reactivity and accessibility [9]. A lotion with Lacticaseibacillus paracasei and Lacticaseibacillus rhamnosus fermented lysates improves skin moisture, strengthens the barrier, and relieves sensitive skin symptoms [10].

In our earlier study, we isolated a new probiotic strain, Bifidobacterium longum subsp. iuvenis (Bl. iuvenis), and found that its lysate had antiphotoaging effects [11]. Nevertheless, the function of Bl. iuvenis in skin wound healing and barrier protection had not been thoroughly investigated. Thus, this study was designed to systematically evaluate the performance of the lysate of Bifidobacterium longum subsp. iuvenis YSG (YSG-BL) in the skin repair process. By conducting a series of in vitro and ex vivo experiments, we showed that YSG-BL could promote cellular scratch repair, dampen inflammatory responses, and mitigate the skin barrier damage triggered by sodium lauryl sulfate (SLS). These results suggest that YSG-BL is a viable option for skin barrier restoration and may have prospects in dermatological and cosmetic applications.

2. Materials and Methods

Cell lines and tissues

Human HaCaT keratinocytes (iCell Bioscience Inc., Shanghai) and mouse NIH-3T3 fibroblasts were cultured in Dulbecco's Modified Eagle Medium (DMEM, 11965092, Gibco) and Roswell Park Memorial Institute 1640 medium (RPMI 1640, C11875500CP, Gibco), respectively. Both media were supplemented with 10% fetal bovine serum (SORFA, China). The artificial skin model, EpiKutis, was procured from BIOCELL BIOTECH (Shaanxi, China).

Wound healing assay

HaCaT cells (5×10^5 cells/well) were seeded in 6 - well plates and incubated at 37°C for 24 h. A linear scratch was created in the confluent cell layer using a 10 μl pipette tip. After washing the wells three times with PBS to remove debris, YSG - BL solutions in serum - free medium were added. Images of the scratched areas were taken at 0 and 18 h using an inverted microscope. The wound closure rate was analyzed quantitatively with ImageJ software.

ELISA assay

The levels of inflammatory cytokines, including interleukin-6 (IL-6) and interleukin-1α (IL-1α), in the cell culture supernatants were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits: the Human IL-6 ELISA Kit (EK106, MultiSciences) and Human IL-1α ELISA Kit (EK101A, MultiSciences).

HaCaT cells (3×10^5) were seeded in 35 mm plates, adhered overnight, treated with 0.5% YSG-BL for 24 h. After replacing supernatants with PBS, cells received 300 mJ/cm² UVB. Then, cells were transferred to fresh DMEM and incubated for another 24 h. Supernatants were collected, and IL-6/IL-1α levels were assayed per kit instructions. Absorbance at 450 nm was read on an Epoch microplate reader (BioTek) for quantification.

Western blot assay

The protein expression of involucrin was assessed via Western blot analysis. Initially, HaCaT cells were seeded in 6 - well plates. After cell adhesion, they were treated with 0.5% YSG - BL for different time periods. Total proteins were extracted from each sample using RIPA lysis buffer. The extracted proteins were resolved by 10% sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS - PAGE) and then transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with 5% skim milk at room temperature for 1 hour. Subsequently, it was incubated overnight at 4°C with primary antibodies against involucrin (83649-5 RR, Proteintech) and glyceraldehyde 3 phosphate dehydrogenase (GAPDH, AC002, ABclonal). After thorough washing with Tris - buffered saline with Tween 20 (TBST) buffer, the membrane was incubated with corresponding secondary antibodies for 1 hour at room temperature. Finally, protein bands were visualized and imaged using the ChemiDoc imaging system (Bio-Rad).

Immunofluorescence microscopy

The expression and subcellular localization of involucrin were investigated using immunofluorescence (IF) assays. HaCaT cells were first seeded on slides in plate and incubated overnight. Subsequently, the cells were treated with 0.5% YSG - BL for an additional 24 hours. The treated cells were fixed with 4% paraformaldehyde in PBS for 30 minutes at room temperature. After being washed three times with PBS, the cells on slides were blocked with a Blocking Buffer (1x PBS/5% normal serum/0.3% Triton X-100) for 60 minutes. This was followed by an overnight incubation at 4°C with the primary antibody against involucrin (83649-5 RR, proteintech). After washing the cells three times with PBS, they were incubated with DyLight 594

AffiniPure Goat Anti Rabbit secondary antibody (FD0129, Fdbio Science) for 2 hours at room temperature. DAPI (FD7229, Fdbio Science) was added for an additional 5 minute incubation. The coverslips were then mounted onto microscope slides, and images were captured using a Zeiss LSM880 confocal microscope. The fluorescence intensity of the stained cells was quantified using ImageJ software.

3D skin model treatment

EpiKutis tissues were carefully inoculated into 6 well plates containing 0.9 ml of EpiGrowth medium. Subsequently, 25 µl of a 0.1% Sodium lauryl sulfate (SLS) solution was applied to the surface of the 3D skin models, and the plates were incubated for 30 minutes. The SLS treated EpiKutis tissues were then exposed to either 5% YSG-BL or 50 µM pirinixic acid (serving as the positive control) and further incubated for 24 hours at 37°C in a humidified atmosphere with 5% CO_2. After the incubation period, the surface of the 3D skin models was gently washed with sterile PBS solution, and any remaining liquid was carefully blotted dry using sterile cotton swabs. The EpiKutis tissues were then fixed in 4% paraformaldehyde for subsequent hematoxylin and eosin (H&E) staining. Microscopic images of the stained tissues were acquired to assess the morphological changes.

3. Results

YSG - BL enhances the migration of HaCaT and NIH - 3T3 cells

The migration of keratinocytes and fibroblasts during the wound healing process is a pivotal event in re - epithelialization, serving as a critical biomarker for evaluating the early phases of wound repair [12]. To elucidate the influence of YSG-BL on cell migration, a wound healing assay was conducted.

As illustrated in Figure 1A, treatment of HaCaT cells with 0.5%, 1%, and 2% YSG-BL for 18 hours significantly enhanced cell migration. In contrast, application of 5% YSG-BL did not elicit a statistically significant increase in HaCaT cell migration. Moreover, incubation of NIH-3T3 cells with 2% and 5% YSG-BL for 18 hours resulted in a notable, statistically significant augmentation of cell motility (Figure 1B).

Collectively, these findings demonstrate that YSG-BL promotes cell migration in a concentration-dependent manner, suggesting its potential therapeutic utility in wound healing and skin barrier repair.

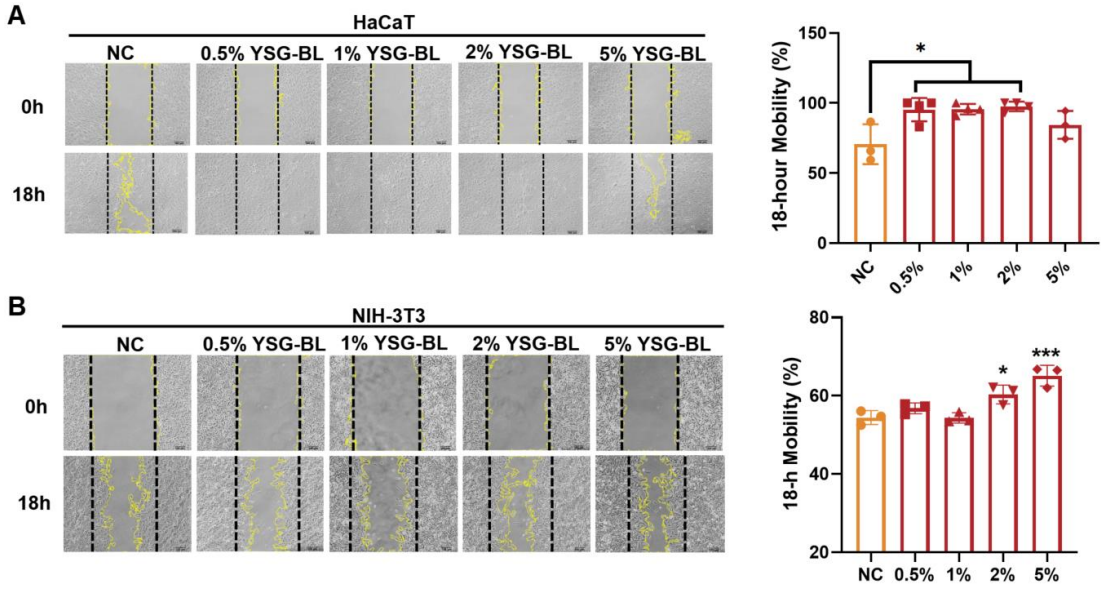

Figure 1. Effects of YSG-BL on on the migration of HaCaT cells (A) and NIH-3T3 cells (B).

YSG - BL mitigates inflammatory responses induced by UVB exposure

To determine whether YSG-BL can attenuate the inflammatory process, a UVB-induced inflammation model was established, and protein levels of IL-1α and IL-6 were quantified using an enzyme linked immunosorbent assay (ELISA). As illustrated in Figure 2, compared with the negative control (NC), UVB treatment led to a substantial increase in the protein levels of IL-1α and IL-6. Conversely, treatment with YSG - BL significantly reduced the elevated levels of IL-1α and IL-6 in UVB - exposed HaCaT cells. These results strongly indicate that YSG-BL effectively suppresses cellular inflammation, which may contribute to the alleviation of cellular senescence.

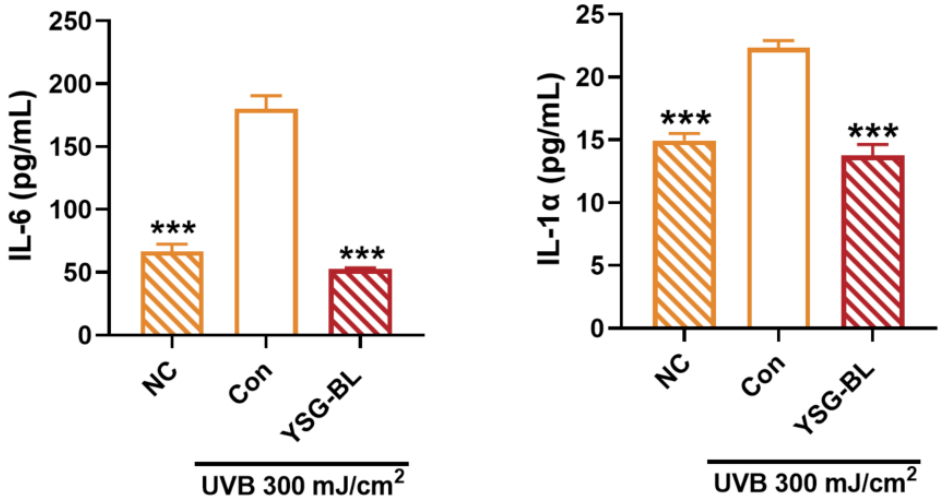

Figure 2. YSG-BL alleviates UVB-induced inflammation. The protein level of IL-6 and IL-1α were show with or without YSG-BL under UVB exposure.

YSG-BL enhances the skin barrier by inducing involucrin protein expression

Involucrin (IVL) is a pivotal marker protein that promotes the terminal differentiation of keratinocytes during the formation of the stratified squamous epithelium [13]. Its expression level is regarded as a critical biomarker for assessing skin barrier integrity and the efficacy of cosmetic interventions. In the present study, Western blot (WB) and immunofluorescence (IF) assays were employed to investigate whether YSG - BL could enhance the skin barrier by upregulating the expression of IVL protein.

The results of the WB analysis demonstrated that treatment with 0.5% YSG - BL led to a significant increase in IVL protein expression in HaCaT cells as early as 120 minutes post treatment as shown as in Figure 3A. Consistent with these findings, the IF results revealed that the fluorescence intensity of IVL was markedly higher in the YSG - BL - treated group compared to the negative control (NC) group (Figure 3B).

Collectively, these data strongly suggest that YSG - BL plays a crucial role in fortifying the skin barrier through the induction of involucrin expression.

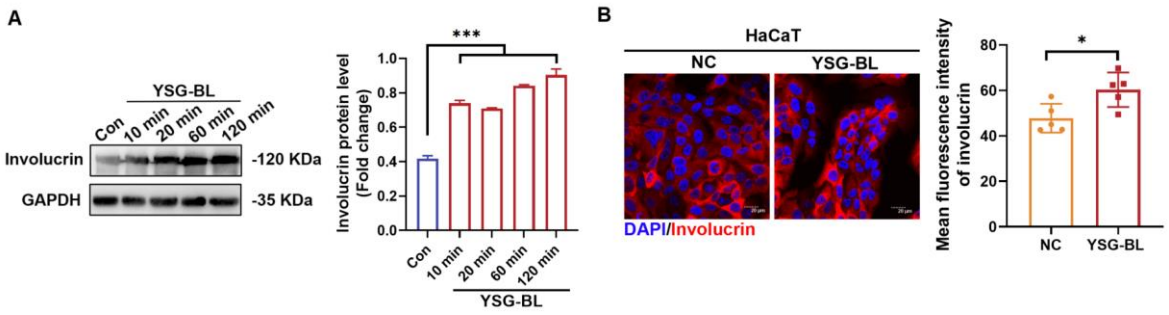

Figure 3. YSG-BL induces the protein expression of involucrin (IVL). A. The protein level of IVL was shown by westen blot at different time slots treated by YSG-BL. B. Immunofluorescence (IF) staining was used to visualize the protein level of IVL 120 minutes after treatment with YSG - BL.

YSG - BL restores the morphology of 3D skin tissue

Sodium lauryl sulfate (SLS), an anionic surfactant, is known to disrupt cell membrane proteins and induce skin irritation, making it a commonly used agent in the evaluation of cosmetic efficacy [14]. In this study, hematoxylin and eosin (H&E) staining was utilized to examine the histomorphological alterations in 3D skin models.

The results showed that, when compared to the negative control (NC) group, treatment of the EpiKutis® 3D skin model with 0.1% SLS caused notable changes. Specifically, the epidermis and stratum corneum became loosened and thickened, accompanied by the formation of vacuoles and substantial tissue damage. In contrast, upon treatment with 5% YSG-BL, the number of vacuoles in the SLS - damaged skin model was significantly decreased. Moreover, both cellular injury and tissue morphology were markedly improved, with the outcomes closely resembling those of the positive control group treated with Pirinixic Acid (Figure 4).

These findings strongly suggest that YSG-BL effectively restores the morphology of damaged skin tissue and possesses potent skin - repairing capabilities.

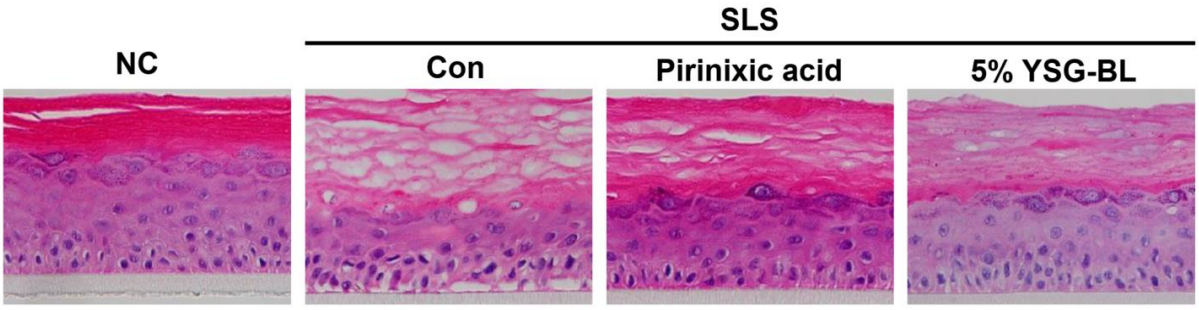

Figure 4. YSG-BL repairs skin morphology in an SDS-damaged skin model

4. Discussion

Probiotics and their fermented products have been widely used in the preparation of oral and topical cosmetics due to their excellent anti-aging, moisturizing or whitening properties [15]. As the most commonly used safe probiotic from human, Bifidobacterium longum and its lysates have been shown to be effective in promoting gut and skin health [6, 9, 11]. In our study, we found that the lysates derived from Bl. Iuvenis YSG can promote repair and regeneration of cell and skin tissue, and reduce the expression of inflammatory cytokines IL-1α and IL-6. This

suggests that YSG-BL has the potential to be developed as soothing and restorative cosmetic products.

Wound healing represents a highly intricate and precisely coordinated biological process through which the body endeavors to reestablish the integrity of damaged or non - viable tissues. Accumulating evidence from numerous studies has underscored the diverse beneficial impacts of probiotics on wound repair [16]. For instance, the topical administration of probiotic strains such as Lactiplantibacillus plantarum, Lactobacillus rhamnosus, and Bifidobacterium longum has been shown to expedite wound healing [17]. Additionally, postbiotics derived from Limosilactobacillus fermentum and Limosilactobacillus reuteri have been reported to mitigate skin inflammation and accelerate the wound - healing process [18]. Our results indicate that YSG-BL promotes scratch repair in keratinocytes and stimulates the expression of intracellular involucrin protein. This action may be instrumental in the initial reconstitution of the epidermal barrier, thereby providing a foundation for subsequent wound healing progression.

Notably, YSG-BL demonstrates the ability to reverse skin barrier damage induced by sodium lauryl sulfate (SLS) exposure. However, the underlying molecular mechanisms by which YSG BL orchestrates skin barrier repair remain largely elusive. Future research endeavors will thus focus on the identification of key active molecules within YSG-BL. This exploration is anticipated to not only facilitate the discovery of novel, small molecule based skin care agents but also contribute to a more comprehensive understanding of the intricate repair mechanisms involved in skin barrier restoration.maged skin model

5. Conclusion

Overall, YSG-BL demonstrates multiple mechanisms of skin barrier repair: keratinocyte migration promotion, UVB-induced inflammation suppression, and involucrin upregulation. Its effectiveness in rescuing SLS-damaged 3D skin models highlights its utility. As a novel probiotic agent, YSG-BL holds great potential for addressing barrier dysfunction and inflammation. Research into its bioactive molecules and mechanisms is essential for future applications.

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