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Limosilactobacillus reuteri CCFM1403 Postbiotic Regulates Skin Barrier Proteins and Reveals Peptidoglycan as Its Key Active Component

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ABSTRACT: The integrity of the skin barrier is essential for maintaining cutaneous homeostasis, and its disruption contributes to various dermatological disorders. Postbiotics, defined as non-viable microbial products with established safety and stability, have attracted growing attention for improving skin health; however, the active components and mechanisms responsible for their barrier-repairing effects remain unclear. This study investigated the postbiotic of *Limosilactobacillus reuteri* CCFM1403 to identify its key bioactive substance and elucidate its role in skin barrier restoration. Using sodium dodecyl sulfate (SDS)-induced HaCaT cell damage and 1-fluoro-2,4-dinitrobenzene (DNFB)-induced murine barrier injury models, the *L. reuteri* CCFM1403 postbiotic significantly improved cell viability (restoring viability from 51.73% to over 80%), upregulated structural and functional barrier proteins, reduced transepidermal water loss (TEWL) to 14.55 g/m²/h, and enhanced skin hydration to 26.67%. Among the isolated fractions, the cell wall exhibited the strongest repair activity, and analysis identified peptidoglycan as the principal active compound. These findings demonstrate that cell wall-derived peptidoglycan is the primary bioactive component mediating the skin barrier-repairing effect of *L. reuteri* CCFM1403, providing mechanistic insight for the development of postbiotic-based functional food.

Key words: Functional food; skin barrier; postbiotic; peptidoglycan

1. Introduction

As the body's first line of defense, the skin plays a vital role in preserving overall health through its barrier function. Its primary functions are to prevent moisture loss, repel harmful external substances and microbial invasion, and regulate systemic or local immune responses^[1]. The skin barrier is primarily made up of the stratum corneum, tight junctions and the basal membrane when it is intact. When compromised, the skin barrier leads to increased transepidermal water loss (TEWL) and reduced stratum corneum hydration. This

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subsequently triggers skin dryness, inflammatory responses and various dermatological conditions [2]. Therefore, it is important to identify safe active substances that can effectively restore skin barrier function in order to maintain skin health.

In recent years, the role of the skin microbiome in maintaining skin homeostasis and barrier function has received a great deal of attention. Dietary interventions incorporating functional components, such as prebiotics, probiotics and medicinal foods, play a crucial role in promoting human health, including skin health [3]. The skin's resident microbiota modulates the differentiation of keratinocytes, the formation of tight junctions, and immune responses by producing metabolic by-products and surface components, and by interacting with host cells. This contributes to the maintenance of barrier homeostasis [4]. Traditional interventions in the field of skin microbiology have predominantly focused on the topical or oral administration of live bacteria (i.e. probiotics). However, these approaches have limitations with regard to stability, safety and survival within the complex environment of the skin [5]. In contrast, postbiotics, which have become more prominent in recent years, are considered a more effective way of regulating skin health thanks to their stability and clearly defined active components [6]. Research has demonstrated that postbiotics derived from the species *Lactobacillus* and *Bifidobacterium* exhibit a variety of bioactive functions. These include ameliorating chronic kidney disease, regulating immunity and improving skin condition [7–9]. However, postbiotics derived from different bacterial strains can vary significantly in their efficacy. Their active constituents and mechanisms of action are still not fully understood.

Of the many postbiotic components, the cell wall and its primary structural component, peptidoglycan, are considered to be key factors in biological activity due to their direct interaction with host pattern recognition receptors [10]. The Gram-positive bacteria peptidoglycan consists of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), which are linked by β -1,4-glycosidic bonds. Four amino acid molecules, in L- and D-forms, are alternately attached to NAM to form the peptide tail. These peptide tails are linked by peptide bridges, and it is the variability of these bridges that is the primary cause of peptidoglycan diversity [11]. For instance, it had been demonstrated that peptidoglycans derived from different *L.* strains exhibit strain-specific immune modulation and intestinal barrier repair properties. However, their role in skin barrier restoration remains understudied [12].

This study used *L. reuteri* CCFM1403 as its subject, with the aim of systematically evaluating the role of its postbiotics and distinct structural components (surface layer proteins, cell wall and protoplast) in skin barrier repair. This study used *L. reuteri* CCFM1403 as its subject, with the aim of systematically evaluating the role of its postbiotics and distinct structural components (surface proteins, cell wall and protoplast) in skin barrier repair.

2. Materials and methods

2.1 Cell lines and bacterial strain

Five strains of *Limosilactobacillus reuteri*—CCFM1403, FSDLZ13M1, FSDLZ12M1, 82 and 92, *Lactiplantibacillus plantarum* FSCDJY93L1 and *Lacticaseibacillus paracasei* FQHXN78L2 were sourced from the Culture Collection of Food Microorganisms (CCFM), maintained at Jiangnan University.

The human epidermal keratinocyte cell line (HaCaT) was acquired from the Cell Bank (Shanghai, China).

2.2 Bacterial fermentation

The bacterial strains were first revived on a modified MRS agar medium composed of yeast extract (5.0 g/L), peptone (10.0 g/L), glucose (20.0 g/L), anhydrous sodium acetate (2.0 g/L), diammonium hydrogen citrate (2.0 g/L), dipotassium hydrogen phosphate (2.6 g/L), manganese sulfate monohydrate (0.25 g/L), magnesium sulfate heptahydrate (0.5 g/L), and Tween-80 (1 mL/L). The pH of the modified MRS medium was adjusted to 6.2–6.4 to sterilization, and the pH remained stable after autoclaving. Incubation was carried out for 48 hours. Subsequently, individual colonies were inoculated into the corresponding modified MRS broth and subcultured for three successive passages, each lasting 16 hours. Cultures from the third passage were subjected to centrifugation at 8000×g for 15 minutes to obtain the bacterial pellet for downstream applications. The bacteria pellet were resuspended in normal saline and pasteurized to obtain postbiotic.

2.3 Cell culture, model and treatment

HaCaT cell was maintained in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Gibco) and antibiotics (ampicillin at 10 U/mL and streptomycin at 10 µg/mL; Biolight Biotechnology Co., Ltd., Shanghai, China). Cell cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO₂.

The groups included control group, model group and treatment group. The cells were seeded in 96 well (1.2×10^4 cells/well) and 6 well (3.6×10^4 cells/well) plates for 36 hours until attachment was completed. The model group and treatment group were exposed to sodium dodecyl sulfate (SDS) solution (15 µg/mL, DMEM, without FBS) for 6 hours, while the control group received DMEM without FBS. After incubation, the cells were washed with PBS; The treatment group received bacterial lysate, while the control group and model group received DMEM without FBS.

2.4 Animal model and treatment

Animal experiments were conducted in two batches using 7-week-old female Balb/c mice, which were maintained under standard housing conditions (12 h light/dark cycle, 21–25 °C, 40–60% humidity). The modeling procedure was identical for both batches, with only the gavage samples differing. All procedures were approved by the Experimental Animal Ethics Committee of Jiangnan University (Approval No. JN.No20231230b1280215[637] and JN.No20241115b0960109[589]) and were performed in accordance with the European guidelines for the protection of laboratory animals. After one week of acclimatization under standard conditions, the experiments were initiated.

The skin barrier disruption model was adapted from the method of Fang, Li^[13]. Briefly, the dorsal hair of each mouse was shaved and depilated to create a 2 cm × 1.5 cm exposed area. A vehicle solution of acetone and olive oil (4:1, v/v) was used to prepare 1-fluoro-2,4-dinitrobenzene (DNFB) solutions at concentrations of 0.5% and 0.2%. At the beginning of week 3, mice were sensitized with 25 µL of 0.5% DNFB applied to the dorsal skin, followed three days later by 25 µL of 0.2% DNFB. Subsequently, 25 µL of 0.2% DNFB was applied every two days until the end of the experiment. Control mice were treated with an equal volume of vehicle solution.

In the first batch, 32 mice were randomly assigned to four groups (n=8 per group), the gavage dose was referred to the previous method^[14]: (1) Blank group: gavaged with 0.2 mL saline; (2) Model group: gavaged with 0.2 mL saline; (3) CCFM1403 postbiotic group: gavaged with 0.2 mL of inactivated CCFM1403 equivalent to 5×10^9 CFU/mL; (4) CCFM1403 live bacteria group: gavaged with 0.2 mL of 5×10^9 CFU/mL CCFM1403 live bacteria.

In the second batch, 40 mice were randomly divided into five groups (n=8 per group): (1) Blank group: gavaged with 0.2 mL saline; (2) Model group: gavaged with 0.2 mL saline; (3) CCFM1403 surface protein group: gavaged with 0.2 mL of surface proteins extracted from 1×10^9 CFU equivalent bacteria; (4) CCFM1403 cell wall group: gavaged with 0.2 mL of cell wall components extracted from 1×10^9 CFU equivalent bacteria; (5) CCFM1403 protoplast group: gavaged with 0.2 mL of protoplasts extracted from 1×10^9 CFU equivalent bacteria. All interventions were administered by gavage for 3 weeks following the acclimation period.

At the end of the experiment, mice were anesthetized with 4% (v/v) isoflurane at an oxygen flow rate of 2 L/min. Blood was collected via retro-orbital puncture, after which the mice were euthanized. Blood samples were centrifuged at 5600 r/min for 15 min at room temperature to obtain serum. Dorsal skin and spleen tissues were collected: part of the skin was fixed in 4% paraformaldehyde for histological analysis, while the remaining portion was homogenized for inflammatory cytokine assays. The spleen was weighed immediately to calculate the spleen index (spleen weight (mg) / body weight (g) × 100%).

2.5 Cell viability assays

HaCaT cells were seeded into 96 well plates with 1.2×10^4 cells/well, and the outer circle of the plate was filled with PBS solution (blank group) to prevent edge effect. After 36 hours of culture, the control group and treatment group (including model group and experimental group) were set up. Blank group: containing cell culture medium and HaCaT cells, without modeling. Model group: containing cell culture medium and HaCaT cells, treated with 15 µg/mL SDS for 6 hours, discarded the supernatant, and then incubated with cell culture medium for 24 hours. Experimental group: containing cell culture medium and HaCaT cells, treated with 15 µg/mL SDS for 6 hours, discarded the supernatant, and then incubated with epigenetic sample for 24 hours. After incubation, 10 µL CCK8 solution was added to each well for 1.5 hours to measure the absorbance (OD) at 450nm. The cell viability was calculated according to the following formula: Cell Viability (%)=(OD treatment group - OD blank group)/(OD control group - OD blank group) × 100%.

2.6 Quantitative real-time RT-PCR (qRT-PCR)

According to the manufacturer's protocol, total RNA from cells was extracted using the FastPure Complex Cell Total RNA Isolation Kit (RC113-C1, Vazyme, Nanjing, China). Total RNA from mice skin was extracted using the FastPure Complex Cell Total RNA Isolation Kit (RC112-C1, Vazyme, Nanjing, China). The purified RNA was subsequently subjected to reverse transcription with the HiScript III RT SuperMix for qPCR (R33301, Vazyme, Nanjing, China) to generate cDNA. Expression analysis of target genes was performed by real-time quantitative PCR using the ChamQ Universal SYBR qPCR Master Mix (Q711-02, Vazyme, Nanjing, China). Relative mRNA abundance was determined by applying the $2^{-\Delta\Delta C_t}$ calculation method. The nucleotide sequences of the primers used are listed in Table 1.

Table1 Gene and primer sequences

Gene	Forward Primer (5'-3')	Reverse Primer (3'-5')
FLG	GCACTCGTCATGCAGAGACTT	GACCCTCGGTTTCCACTGT
IVL	GACTGCTGTAAAGGGACTGCC	CATTCCCAGTTGCTCATCTCTC
LOR	GCTCTGTCTGCGGCTACTCTG	GTGACCTGCTGCGAGGAGAC
ZO-1	CAACATACAGTGACGCTTCACA	CACTATTGACGTTTCCCCACTC
OCLUDDIN	GACTTCAGGCAGCCTCGTTAC	GCCAGTTGTGTAGTCTGTCTCA
CLDN	TCTGGCTATTTTAGTTGCCACAG	AGAGAGCCTGACCAAATTCGT
Laminin	GAGGGGCCTGTTATCCACCT	CTGCCACTGTCCATATTGGGT
Fibronectin	ATGTGGACCCCTCCTGATAGT	GCCCAGTGATTTCAGCAAAGG
GAPDH(cell)	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
GAPDH(mice)	TGAGTGGCAAAGTGGAGAT	TTTGCCGTGAGTGGAGTCAT

2.7 Enzyme-linked immunosorbent assay (ELISA)

For protein analysis, 50 mg of skin tissue was homogenized in 450 μ L PBS using a high-throughput tissue homogenizer operated at 65 Hz for ten 30-s cycles. Homogenates were centrifuged (3000 rpm, 15 min, 4 °C), and the supernatant fractions were collected. Expression levels of barrier-related proteins, including FLG, IVL, LOR, ZO-1, OCLUDDIN, CLDN1, as well as HIS, were quantified using commercial assay kits (Shanghai Enzyme-linked Biotechnology Co., Ltd.). Serum IgE levels were also measured to evaluate systemic immune responses.

2.8 Measurement of TEWL and water content

On the 21st day, TEWL and skin moisture levels were determined under chloral hydrate anesthesia. Measurements were carried out with a Cutometer DUAL MPA580 using Tewameter Hex and Corneometer CM825 probes, with data recorded perpendicularly at three separate skin locations for each mouse.

2.9 Histopathology

Dorsal skin tissues (1 cm²) were fixed for 24 h in general-purpose tissue fixative (G1101, Servicebio, Shanghai, China), embedded in paraffin, and sectioned. After routine dewaxing and rehydration, the sections were stained with Hematoxylin and Eosin (H&E). Epidermal thickness and other histopathological features were analyzed using a digital section scanner, with ImageJ software employed for quantitative evaluation.

2.10 Immunofluorescence

Paraffin-embedded tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval. Following PBS washes, nonspecific binding was blocked with 3% BSA. Sections were then incubated overnight at 4 °C in a humidified chamber with primary antibodies against fibronectin and laminin, followed by incubation at room temperature in the dark with appropriate fluorescent secondary antibodies (Alexa Fluor 488-conjugated goat anti-rabbit IgG). Nuclei were counterstained with DAPI, and autofluorescence quenching was applied when necessary. Finally, the sections were mounted with antifade medium, and images were acquired using a fluorescence microscope equipped with standard filter sets.

2.11 Bacterial component extraction

The extraction procedures for bacterial surface layer proteins, cell wall fractions, peptidoglycan and protoplasts were performed as follows.

2.11.1 Extraction of surface layer protein

Surface proteins were extracted following a modified protocol ^[15]. Briefly, bacterial cells were harvested at the logarithmic growth phase by centrifugation, washed with deionized water, and resuspended in 5 M LiCl. After incubation at 37 °C with shaking for 1 h, the suspension was centrifuged, and the supernatant was filtered through a 0.45 µm membrane. The filtrate was dialyzed against deionized water using a 15 kDa cut-off membrane for 24 h, followed by SDS-PAGE confirmation and lyophilization for storage.

2.11.2 Extraction of cell wall

Cell wall components were extracted following with minor modifications ^[16]. Bacterial pellets were boiled in 5% SDS (50 mM MES, pH 5.5), and the insoluble fractions were repeatedly washed with deionized water until free of SDS. The pellet was then sequentially treated with pronase E (2 mg/mL), trypsin (200 µg/mL), and DNase/RNase (50 µg/mL each) under appropriate conditions, followed by incubation in 2% SDS to inactivate residual enzymes. After repeated washing, the final insoluble cell wall fraction was collected and stored at −20 °C until use.

2.11.3 Extraction of peptidoglycan

Peptidoglycan was purified according to a modified protocol ^[17]. Briefly, crude cell wall fractions were sonicated in distilled water and sequentially treated with Benzonase (50 U) and trypsin (50 µg/mL) at 37 °C with shaking. The mixture was boiled to inactivate enzymes, centrifuged, and the pellet was washed thoroughly with distilled water. To remove wall teichoic acids and glycophosphate, the pellet was incubated in 1 M HCl at 37 °C for 4 h, followed by repeated washing until the pH of the supernatant reached 4–5. The resulting peptidoglycan fraction was resuspended in distilled water, lyophilized, and stored at −20 °C until use.

2.11.4 Extraction of protoplast

Protoplasts were prepared according to a modified protocol ^[18]. Briefly, bacterial pellets were resuspended in SMM buffer (0.02 M Hepes, 1 mM MgCl₂, 0.5 M lactose, pH 6.4) and treated with lysozyme

(100 mg/L) at 37 °C overnight. The reaction was terminated with fresh SMM, and protoplasts were collected by gentle centrifugation, washed, and resuspended in SMM for subsequent use.

2.12 UV–Vis spectral scanning

The peptidoglycan samples were subjected to UV full-wavelength spectral scanning in the range of 200–600 nm to examine the presence of an absorption peak at 280 nm, thereby excluding potential interference from nucleic acids.

2.13 FT-IR spectroscopy

2mg peptidoglycan sample were placed in the Fourier transform infrared spectrometer and scanned in the range of 4000–500 cm⁻¹, followed by analysis after baseline correction.

2.14 statistical analysis

Experiments were repeated three times. Statistical analyses were performed using Origin and SPSS 22.0. Differences among groups were evaluated by one-way or repeated-measures ANOVA with Tukey's post hoc test, and significance was set at $P < 0.05$. Results are shown as mean $\pm$ SD.

3 Results

3.1 CCFM1403 postbiotics enhanced HaCaT cell viability

The functional characteristics of the same lactic acid bacteria may be significantly different at the strain level. Therefore, different *L. reuteri* strains were selected as functional control strains to determine whether the observed biological activities were strain specific rather than general characteristics of the species. To evaluate the reparative effects of different postbiotic components on damaged HaCaT cell viability, we employed the CCK-8 assay to assess cellular activity (Figure 1A). Results demonstrated that compared to the blank control group, cell viability in the model group decreased significantly to 51.73%, indicating successful establishment of the SDS-induced damage model. Among the interventions with different bacterial strains, the cell group (CCFM1403-L) and fermentation supernatant group (CCFM1403-Q) of *L. reuteri* CCFM1403 enhanced HaCaT cell viability. Their recovery levels were markedly superior to those of the corresponding treatment groups for *L. reuteri* FSDLZ12M1, FSDLZ13M1, 82, and 92. Notably, only CCFM1403-L restored cellular activity to over 80%, exhibiting a highly significant difference compared to the model group.

In summary, the postbiotic from CCFM1403 significantly improved the viability of HaCaT cells following SDS-induced damage, indicating its potent cell-repairing effects and demonstrating potential application value in interventions for skin barrier impairment.

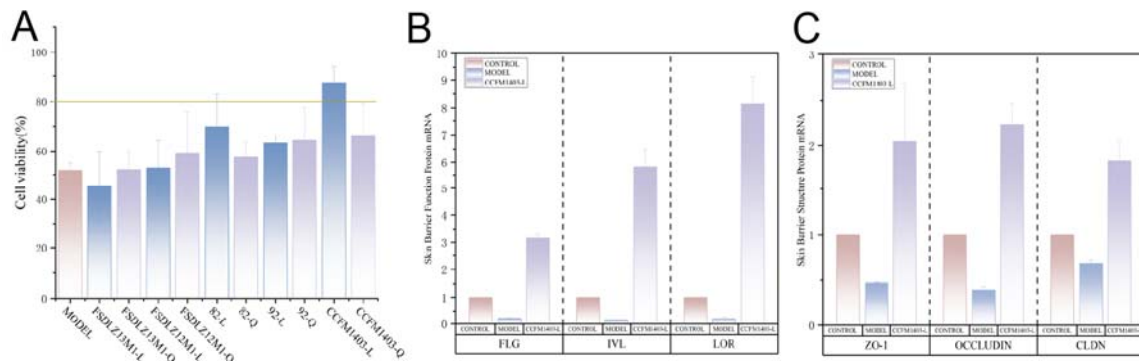


Figure 1 The effects of postbiotics on SDS-injured HaCaT cells. (A) Cell viability after treatment with postbiotic lysate (-L) and fermentation supernatant (-Q). (B) Skin barrier function protein mRNA. (C) Skin barrier structure protein mRNA. ‘-L’ represents lysate; ‘-Q’ represents fermentation supernatant.

3.2 CCFM1403 postbiotics enhanced the gene expression of barrier proteins in injured HaCaT cells

To investigate the effects of CCFM1403 postbiotics on barrier-related gene expression in injured HaCaT, we assessed mRNA levels of skin barrier function proteins (FLG, IVL, LOR) and barrier structural proteins (ZO-1, Occludin, Claudin) (Figure 1B&C). Results revealed that compared with the control group, the expression of various barrier protein genes in the model group was significantly reduced. Following intervention with the CCFM1403 bacterial cell group (CCFM1403-L), mRNA levels of FLG, IVL, and LOR were significantly upregulated, with IVL and LOR expression restored to several-fold levels of the model group (5.8-fold and 8.14-fold, respectively). Concurrently, CCFM1403-L elevated ZO-1, Occludin, and Claudin to 2.04-fold, 2.23-fold, and 1.83-fold, respectively, all significantly higher than the model group, indicating its ameliorative effect on the gene expression of barrier structural proteins.

Collectively, these results demonstrate that the CCFM1403 postbiotic effectively enhances the gene expression of barrier function proteins and barrier structural proteins in post-injury HaCaT cells, suggesting its potential to strengthen skin barrier integrity and repair functions.

3.3 Oral administration CCFM1403 postbiotic improved the injured mice skin apparent condition and barrier function

To further evaluate the skin barrier repair effects of CCFM1403 postbiotic, we assessed the epidermal condition, histological alterations, and barrier function indicators of dorsal skin in mice (Figure 2). As shown in Figure 2A, compared with the control group, dorsal skin in the model group exhibited marked erythema, desquamation, and damage. Following CCFM1403 intervention, skin appearance markedly improved, particularly in the postbiotic group (CCFM1403-S), where erythema and lesions were substantially reduced, approaching the normal group's appearance. The live bacteria group (CCFM1403-H) demonstrated comparatively weaker improvement effects. Histological findings (Figure 2B) further corroborated these observations. The model group exhibited markedly thickened epidermis accompanied by parakeratosis and inflammatory cell infiltration. In contrast, CCFM1403-S intervention substantially mitigated epidermal hyperplasia, restoring skin architecture to near-control levels. CCFM1403-H also demonstrated some improvement, though less pronounced than the heat-killed bacterial group. Skin barrier function testing

(Figure 2C) revealed significantly elevated TEWL and markedly reduced skin hydration in the model group following DNFB treatment. Both parameters improved following CCFM1403 intervention. Specifically, the CCFM1403-S group exhibited significantly reduced TEWL and markedly increased skin hydration ($P < 0.01$), demonstrating superior restorative efficacy compared to the CCFM1403-H group.

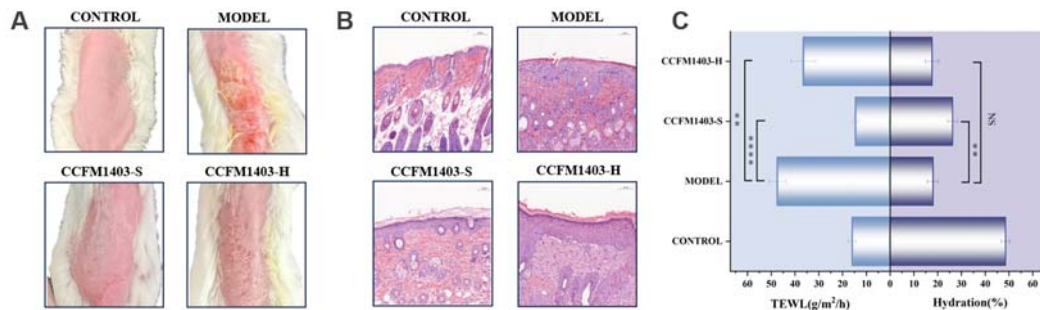


Figure 2 The effect of CCFM1403 postbiotic and live bacteria on mice skin barrier. (A) Skin appearance of mice, (B) H&E staining images, (C) Skin TEWL and hydration. ‘-S’ represents postbiotic. ‘-H’ represents live bacteria. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

In summary, the CCFM1403 postbiotic effectively improved the apparent skin condition and barrier function in mice, with the postbiotic (CCFM1403-S) demonstrating superior restorative effects compared to the live bacteria (CCFM1403-H).

3.4 Oral administration CCFM1403 postbiotic increased the injured mice skin barrier protein content

To evaluate the restorative effect of CCFM1403 postbiotic on skin barrier function, we assessed the expression levels of key barrier proteins. Experimental results demonstrated that, compared with the control group, the content of all detected barrier proteins in the skin of mice in the model group was significantly reduced. The regulatory effects of each intervention group on barrier protein expression are shown in Figure 3A.

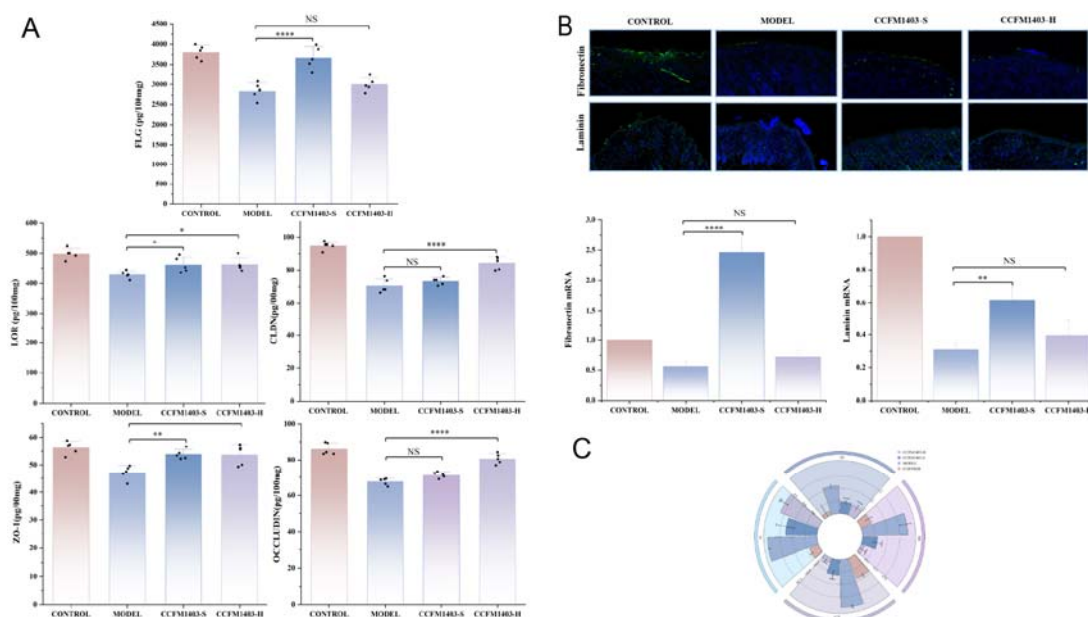


Figure 3 The effects of CCFM1403 postbiotic and live bacteria on skin barrier integrity, basal lamina structure, and immune homeostasis in mice. (A) skin barrier function and structure protein content. (B) skin basal layer protein content. (C) mice immune abnormalities ‘-S’ represents postbiotic. ‘-H’ represents live bacteria. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

In the expression of skin barrier-related genes, CCFM1403-S exhibited the most pronounced upregulation of FLG, with expression levels approximately 1.29-fold higher than the model group, significantly exceeding the 1.06-fold increase observed in the CCFM1403-H group. Regarding LOR, the CCFM1403-S group also demonstrated stronger repair effects, with levels approximately 1.08 times those of the model group, whereas the CCFM1403-H group remained nearly equivalent to the model group, showing no significant difference. Regarding ZO-1 expression, the CCFM1403-S group exhibited an approximately 1.14-fold upregulation compared to the model group, slightly outperforming the 1.13-fold increase observed in the CCFM1403-H group. Regarding Occludin and Claudin, expression of both increased in both groups. Specifically, CCFM1403-S elevated these to 1.06-fold and 1.04-fold that of the model group respectively, whereas CCFM1403-H reached 1.19-fold, indicating more pronounced effects of live bacterial intervention on these two indicators.

In summary, the CCFM1403 postbiotic effectively promotes the expression of core skin barrier proteins. Notably, the inactivated strain CCFM1403-S demonstrated significantly superior efficacy to the live strain in upregulating the key proteins FLG and LOR, highlighting the unique potential of postbiotics in restoring skin barrier function.

3.5 Oral administration CCFM1403 postbiotic increased the injured mice skin basal layer protein content and gene expression

To investigate the effects of the postbiotic CCFM1403 on the structure of the skin basal membrane, we examined the expression of laminin and fibronectin at both the protein and mRNA levels through immunofluorescence staining and qRT-PCR, respectively.

As depicted in Figure 3B, immunofluorescence staining clearly demonstrated that, compared to the control group, the fluorescence signals for laminin and fibronectin in the basement membrane region of the model group mice were markedly diminished. Following intervention with CCFM1403-S, the fluorescence intensity of both proteins was significantly enhanced, with this effect being superior to that observed in the CCFM1403-H group. Gene expression analysis corroborated these protein-level alterations, revealing markedly lower mRNA expression levels for laminin and fibronectin in the model group relative to controls. Following CCFM1403-S intervention, transcription levels for both genes were significantly upregulated, exhibiting a substantially greater increase than in the CCFM1403-H-treated group.

In summary, CCFM1403 postbiotics not only significantly increased the deposition of laminin and fibronectin in the skin basement membrane but also efficiently promoted their synthesis at the transcriptional level, with both effects surpassing those observed in the live bacteria treatment.

3.6 Oral administration CCFM1403 postbiotic improved the inflammatory response in mice

To evaluate the systemic and local ameliorative effects of CCFM1403 postbiotics on inflammatory responses in model mice, we measured skin histamine, skin IL-4, serum IgE levels, and spleen indices. As shown in Figure 3C, both CCFM1403-S and CCFM1403-H demonstrated significant efficacy in reducing skin

histamine and IL-4 two key indicators of cutaneous inflammation. Specifically, CCFM1403-S reduced HIS and IL-4 to 11.91 ng/100mg and 185.62 pg/100mg, respectively, while CCFM1403-H lowered them to 11.21 ng/100mg and 174.66 pg/100mg. Furthermore, regarding systemic inflammation, CCFM1403-S significantly reduced both serum IgE levels and the spleen index, whereas CCFM1403-H only decreased serum IgE levels without affecting the spleen index. Specifically, serum IgE levels in the CCFM1403-S and CCFM1403-H groups were 1.16 and 1.15, respectively, both significantly lower than those in the model group. In contrast, the spleen index was significantly reduced in the CCFM1403-S group (0.48) but not in the CCFM1403-H group (0.55).

In summary, both postbiotic and live bacterial strains of CCFM1403 ameliorate inflammatory responses induced by skin barrier damage in mice to varying degrees.

3.7 CCFM1403 cell wall component enhanced HaCaT cell viability.

Through the results of the aforementioned experiments, we observed that the CCFM1403 postbiotic demonstrated superior efficacy in skin barrier repair compared to the live bacterial strain. Consequently, to identify the postbiotic components responsible for the efficacy of *L. reuteri* CCFM1403, we performed stepwise isolation of the bacterial cells. This yielded, in order of structural composition from outer to inner layers, surface layer proteins, cell wall components, and protoplast fractions. SDS-PAGE analysis revealed surface proteins predominantly distributed within the 25–71 kDa range, with a protein concentration of 43 mg/mL. Microscopic observation indicated that protoplasts exhibited regular spherical morphology after cell wall removal, whereas cell walls appeared as irregular flake-like fragments (Figure 4A), suggesting successful separation. Furthermore, cellular assays established a safe dosage of 1.25% for all fractions (Figure 4B). Subsequent testing evaluated the effects of different fractions on the viability of damaged HaCaT cells (Figure 4B). Results showed the cell wall fraction exhibited the most pronounced reparative effect, restoring cellular activity to 91.01%—significantly higher than the model group and exceeding the 80% efficacy threshold. In contrast, activity levels following treatment with the protoplast and surface protein fractions reached 78.30% and 78.09% respectively, representing improvement over the model group but failing to attain effective thresholds.

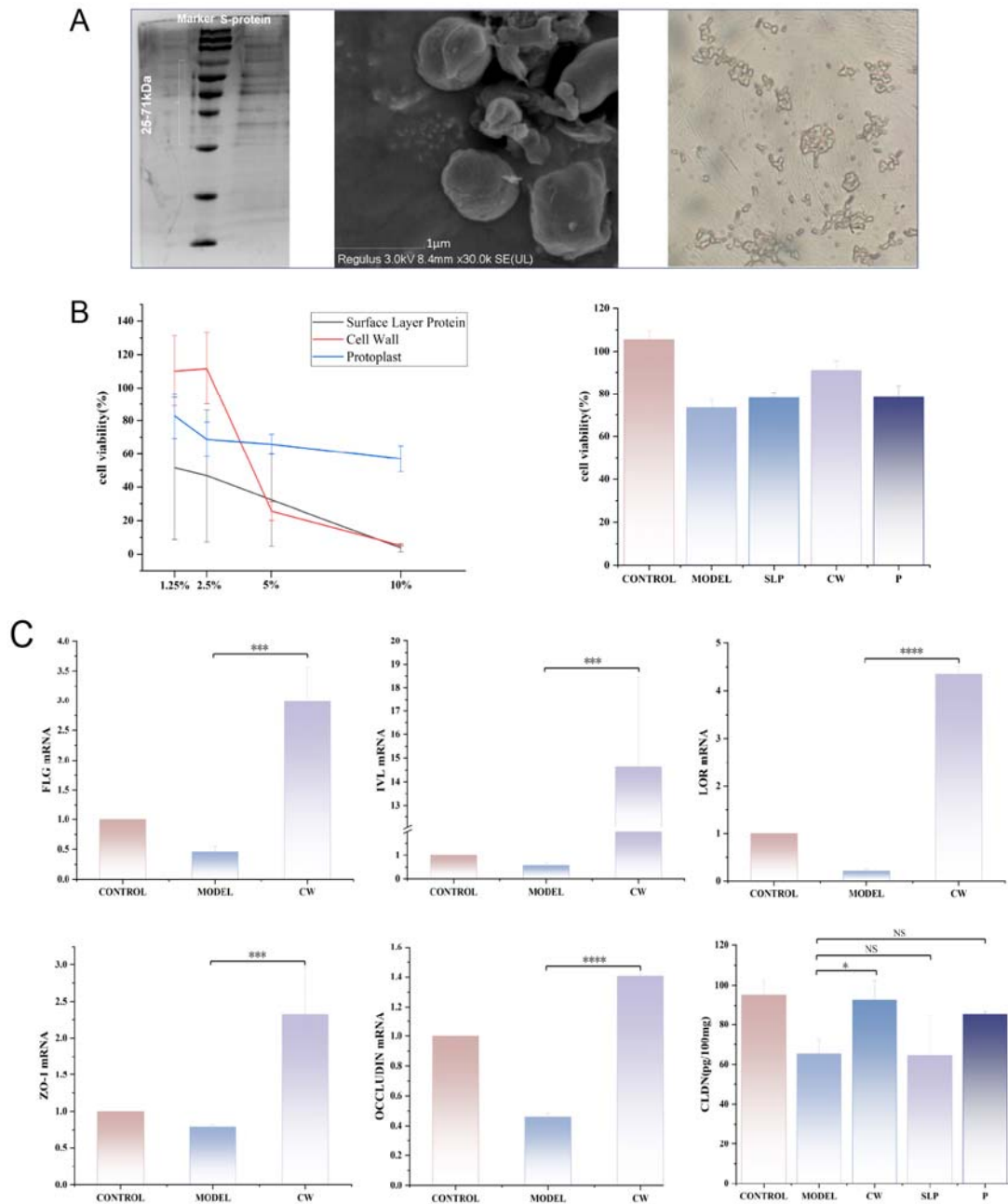


Figure 4 Separation and characterization of CCFM1403 postbiotic components and their effects on HaCaT cell viability and skin barrier-related gene expression. (A) surface layer protein; protoplast transmission electron micrograph; cell wall optical micrograph; (B) cytotoxicity of different components on HaCaT cells; effects of components on the viability of SDS-injured HaCaT cells; (C) effects of cell wall components on skin barrier protein mRNA expression levels. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

In summary, the primary bioactive components of CCFM1403 postbiotics are concentrated within the cell wall, playing a pivotal role in promoting HaCaT cell activity recovery and skin barrier repair. This provides a foundation for subsequent mechanistic investigations.

3.8 CCFM1403 cell wall component increased gene expression of barrier proteins in injured HaCaT cells

To investigate the regulatory effects of CCFM1403 cell wall components on skin barrier-related genes, we assessed the gene expression levels of barrier function proteins and structural proteins in HaCaT cell (Figure 4C). Specifically, FLG expression reached 2.99, representing 6.5-fold that of the model group

(0.46); LOR expression was 3.58, significantly higher than the model group's 0.20; IVL expression reached 15.74, substantially exceeding the model group's 0.58. Furthermore, cell wall components differentially upregulated barrier structural protein-related genes ZO-1, Occludin, and CLDN. Compared to the model group, the relative mRNA expression levels of ZO-1, Occludin, and CLDN in the cell wall group increased from 0.79, 0.46, and 0.52 to 2.33, 1.41, and 0.91, respectively. The cell wall components most significantly promoted the expression of ZO-1 and Occludin genes, with their expression levels reaching 2.9 and 3.5 times that of the model group, respectively. In contrast, although CLDN gene expression was also upregulated (to 1.74 times that of the model group), the magnitude of this increase was markedly lower than that observed for ZO-1 and Occludin.

In summary, cell wall components exert a regulatory effect on the gene expression levels of barrier function proteins and structural proteins.

3.9 Oral administration CCFM1403 cell wall improved the injured mice skin apparent condition and barrier function

To validate the conclusions from in vitro studies and evaluate the in vivo effects of the postbiotic components in CCFM1403, we conducted animal experiments. Skin barrier function was assessed through TEWL, stratum corneum water content, and histopathological examination (Figure 5A-B). Following intervention with different postbiotic components, the cell wall (CW) group demonstrated the most pronounced effects. The CW group exhibited a marked reduction in TEWL and a significant recovery in stratum corneum water content. Macroscopically, both erythema and desquamation showed marked improvement. Histological examination revealed epidermal thickness restored to near-normal levels, with reduced inflammatory response. In contrast, although the superficial layer protein (SLP) and protoplast (P) intervention groups improved skin appearance to some extent, their TEWL and hydration levels showed no significant difference compared to the model group. Histological sections still revealed epidermal thickening and abnormal keratinisation, indicating limited repair efficacy.

In summary, the oral administration of the CCFM1403 cell wall fraction demonstrated the most pronounced efficacy in enhancing skin barrier function, further confirming its status as a key active component.

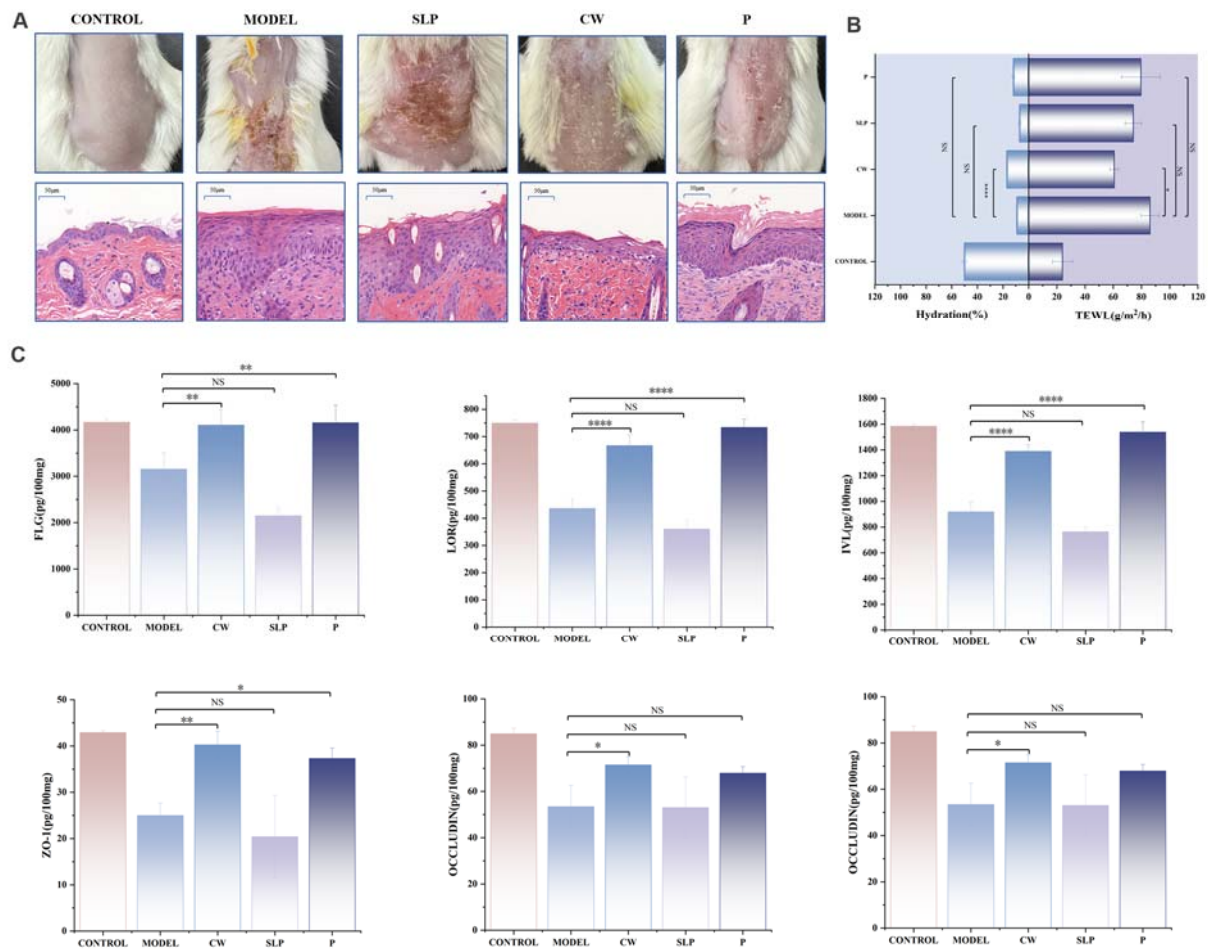


Figure 5 Effects of different CCFM1403 postbiotic components on the skin barrier integrity and barrier protein expression in mice. (A) Skin appearance of mice; (B) H&E-stained skin sections; (C) TEWL and skin hydration; (D) skin barrier protein content.

3.10 Oral administration CCFM1403 cell wall increased the injured mice skin barrier protein content

To evaluate the regulatory effects of the postbiotic components in orally administered CCFM1403 on skin barrier proteins, we assessed the expression levels of key barrier function proteins (FLG, LOR, IVL) and barrier structural proteins (ZO-1, CLDN, Occludin). Regarding barrier functional proteins (Figure 5D), FLG content in the model group (3154.83 pg/100mg) was significantly reduced compared to the blank group (4169.17 pg/100mg). The cell wall intervention group exhibited a significant rebound in FLG content (4106.73 pg/100mg) compared to the model group, with the protoplast group (4157.81 pg/100mg) showing a similar effect. Conversely, the surface protein group (2148.19 pg/100mg) further suppressed FLG expression. Regarding LOR and IVL, both the cell wall group (LOR: 667.04 pg/100mg; IVL: 1389.52 pg/100mg) and the protoplast group (LOR: 733.85 pg/100mg; IVL: 1538.22 pg/100mg) significantly increased their levels, whereas the surface protein group showed no improvement. Regarding barrier structural proteins, cell wall intervention significantly increased the expression of ZO-1 (40.25 pg/100mg), CLDN (92.70 pg/100mg), and Occludin (71.48 pg/100mg). Protoplasts only enhanced ZO-1 expression (37.32 pg/100mg), whilst surface proteins showed no significant effect on any of the three.

Results indicate that the CCFM1403 cell wall is a key active component for improving skin barrier function, effectively promoting the expression of both barrier function proteins and structural proteins. Conversely, surface proteins not only prove ineffective but may even inhibit their synthesis.

3.11 Peptidoglycan in CCFM1403 cell wall component is one of the key substances for repairing the skin barrier

This study found in preliminary results that the cell wall component of CCFM1403 demonstrated the most effective repair of the skin barrier. Given that peptidoglycan constitutes the primary structural component of the cell wall, we further investigated whether it serves as the key active substance, using peptidoglycans from *L. paracasei* and *L. plantarum* as controls. Following extraction and purification, ultraviolet full-wavelength scanning revealed no nucleic acid absorption peaks at 260 nm for all three samples, indicating complete removal of nucleic acid residues from the purified peptidoglycan preparations (Figure6A). Infrared spectroscopy further confirmed the characteristic structural features of peptidoglycan, detecting multiple typical absorption peaks within the 4000–500 cm^{-1} range (Figure6B). For instance: absorption peaks characteristic of β -1,4 glycosidic bonds appeared at 892.10 cm^{-1} , 910.77 cm^{-1} , and 897.18 cm^{-1} , respectively. At 1375.85 cm^{-1} , 1373.05 cm^{-1} and 1374.56 cm^{-1} , respectively, correspond to the asymmetric bending vibrations of CH_3 groups linked within the polypeptide chain. Peaks at 3265.03 cm^{-1} , 3275.22 cm^{-1} , and 3270.13 cm^{-1} , respectively, indicate polypeptide segments connected to the N-terminal amino group. These results indicate that the extracted peptidoglycan possesses an intact structure consistent with expectations.

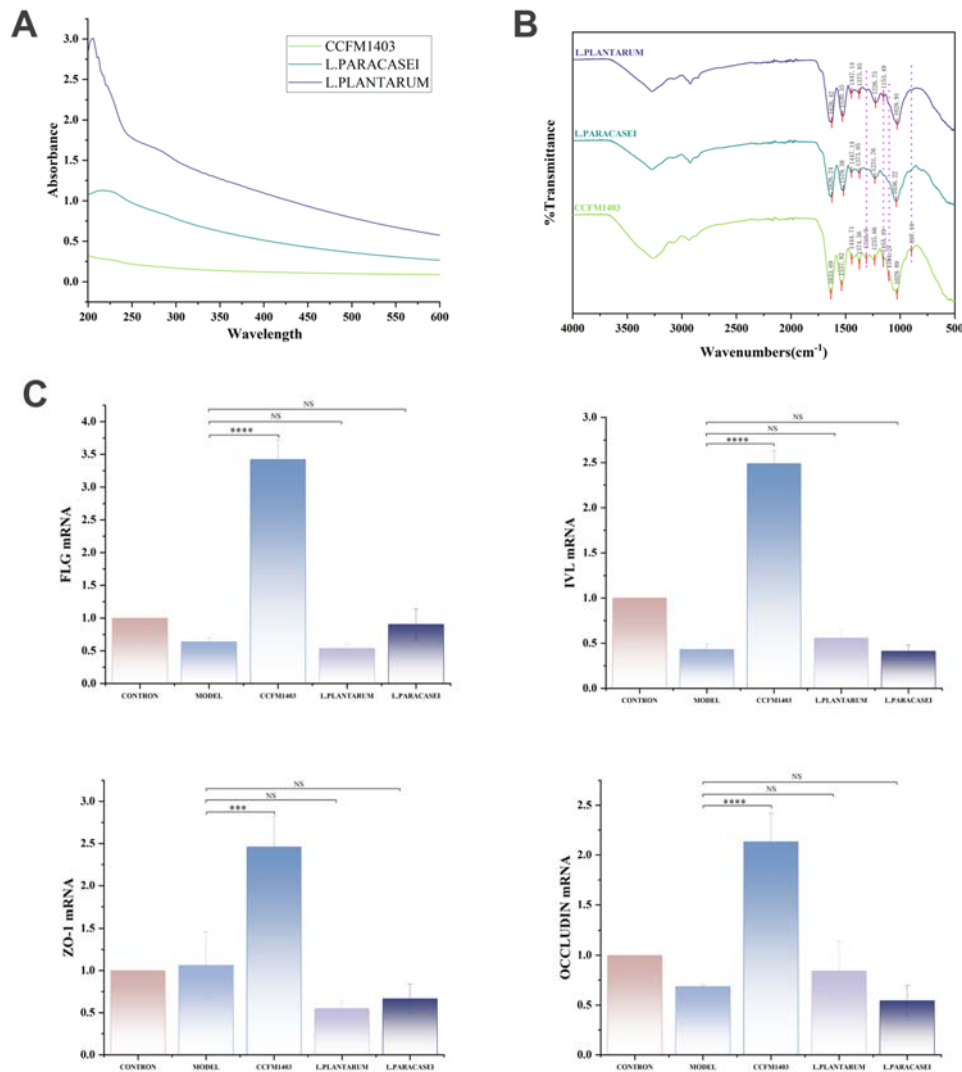


Figure 6 Characterization of different *Lactobacillus* peptidoglycans and effects on skin barrier protein mRNA. (A) UV wavelength scanning, (B) infrared spectrum, (C) skin barrier protein mRNA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Although peptidoglycans exhibit a conserved glycopeptide structure, strain-dependent differences in peptide chain composition and linkage configuration can modulate their physicochemical properties and biological functions. Consistent with this, functional validation demonstrated that CCFM1403 peptidoglycan significantly upregulated the expression of skin barrier proteins FLG and IVL, as well as tight junction-associated proteins Occludin and ZO-1, whereas peptidoglycans from *L. paracasei* and *L. plantarum* did not exhibit comparable effects (Figure 6C). In summary, the peptidoglycan within the CCFM1403 cell wall constitutes a key active ingredient for repairing the skin barrier, with its distinctive structural characteristics potentially conferring unique biological functions.

4. Discussion

In our research, we identified a postbiotic derived from *L. reuteri* CCFM1403 that has been shown to repair the skin barrier. Through a combination of cell and animal experiments, we elucidated the basis of its primary active substance and systematically evaluated its restorative effects. The results showed that CCFM1403 postbiotic had significant restorative effects in both the HaCaT cell damage model and the

DNFB-induced mouse skin damage model. What's more, cell wall components were found to be the most potent, ultimately identifying peptidoglycan as the key bioactive substance.

The integrity of the skin barrier function depends heavily on the coordinated expression of relevant proteins. FLG, LOR and IVL are key proteins synthesised during keratinocyte differentiation that are essential for maintaining the structure and function of the stratum corneum. A reduction or absence of these proteins leads to dyskeratosis and impaired skin barrier function, and is associated with various skin diseases, such as atopic dermatitis and psoriasis^[19]. Concurrently, certain structural proteins—such as ZO-1, Occludin, and Claudin—play a vital role in maintaining the barrier structure between keratinocytes. They prevent excessive TEWL and impede the entry of exogenous allergens or pathogens^[20]. Furthermore, keratinocytes, as the primary constituent cells of the skin barrier, exhibit diminished activity that not only impairs cellular proliferation and differentiation capabilities but also directly affects stratum corneum formation and the synthesis of associated barrier proteins. This study utilised a cell model to demonstrate that CCFM1403 postbiotic significantly restored the viability of HaCaT cells following SDS-induced damage, while markedly upregulating the expression of barrier function proteins (FLG, IVL, LOR) and tight junction proteins (ZO-1, Occludin, Claudin). This finding is consistent with existing research indicating that postbiotics promote keratinocyte differentiation and barrier protein synthesis^[21]. For instance, postbiotics from *Lactobacillus plantarum* have been shown to improve skin barrier integrity by increasing the expression of FLG, LOR proteins^[22]. In comparison, the CCFM1403 postbiotic exhibited a more pronounced ability to restore HaCaT cell viability and upregulate barrier-associated proteins among the tested strains, suggesting a distinct strain-specific advantage. More importantly, the restorative effects of CCFM1403 postbiotic were not only significant but also markedly superior to other control strains in promoting cellular activity recovery, suggesting that its restorative effects exhibit strain specificity. Further animal studies corroborated this finding at the systemic level. The oral administration of the CCFM1403 postbiotic has been demonstrated to significantly improve skin barrier function in mice with DNFB-induced damage. Its improvement is characterised by a reduction in TEWL, an increasing in skin hydration, and a decrease in epidermal thickening and inflammatory cell infiltration at the histological level.

Furthermore, this study also found that the CCFM1403 postbiotic significantly increased the content of skin barrier function proteins and promoted the deposition and transcriptional upregulation of key basal membrane proteins—fibronectin and laminin. The skin basal membrane, which serves as a structural foundation between the epidermis and dermis, provides attachment support for keratinocytes and participates in establishing cell polarity, signal transduction, and maintaining barrier stability^[23]. Consequently, its restoration is of significant importance for the overall repair of the skin barrier. The study by Port, Andelic^[24] similarly confirmed that interactions between ECM components, such as laminin and fibronectin, are crucial for maintaining skin homeostasis.

It is noteworthy that in animal studies, the repair effects of the postbiotic group were superior to those of the live bacterial group. This finding is consistent with the conclusions of recent research, which suggests that

live bacteria are not essential for efficacy, and that inactivated bacterial cells and their components offer greater advantages in terms of stability and safety [25]. This discovery further highlights the potential of postbiotics in skin health interventions. To identify the key bioactive components of CCFM1403 postbiotic, this study performed stepwise separation of the microbial structure. The results indicate that its restorative effects are primarily concentrated in the cell wall region, with peptidoglycan—the principal structural component of the cell wall—playing a central role in this process. *Lactobacillus plantarum* and *Lactobacillus paracasei* were used as control strains to evaluate whether the biological activities were related to specific bacterial peptidoglycans rather than the common properties shared by all *Lactobacillus* species. Compared with the peptidoglycans of *L. paracasei* and *L. plantarum*, the peptidoglycan of CCFM1403 exhibits stronger activity in promoting barrier-related gene expression. It significantly upregulates the expression of barrier function proteins and structural proteins, demonstrating a pronounced strain-specific effect. Furthermore, studies have reported that peptidoglycan can be detected in the blood of both humans and mice, subsequently affecting cells throughout the body. It plays a crucial role in regulating inflammation and systemic metabolic functions [26]. In addition, peptidoglycan fragments can be recognized by intestinal cells through receptors such as NOD1/2 and NLRP3/nlrp1, and then regulate the systemic inflammatory response. NOD1 and NOD2 are the earliest cytoplasmic receptors [27,28].

The peptide composition within peptidoglycans maybe different among bacterial strains. These structural differences may influence their spatial conformation and binding capacity with host receptors, thereby leading to distinct biological effects. Compared with the peptidoglycans of *L. paracasei* and *L. plantarum*, the peptidoglycan of *L. reuteri* CCFM1403 exhibits stronger activity in promoting barrier-related gene expression. It significantly upregulates the expression of barrier function proteins and structural proteins, demonstrating a pronounced strain-specific effect. These differences may result in varying binding capacities between peptidoglycans and host receptors, thereby activating distinct pathways and ultimately inducing different biological responses. Therefore, the specific peptidoglycan structure of *L. reuteri* CCFM1403 may constitute a crucial material basis for its regulation of keratinocyte function, promotion of barrier-associated protein expression, and overall skin barrier repair. What's more, compared with live probiotics, postbiotics possess advantages in stability, storage, and safety, making them more suitable for industrial development and commercialization. Given its significant efficacy in promoting skin barrier repair and regulating inflammatory responses, the CCFM1403 postbiotic may serve as a promising functional ingredient for oral skin-health products, including functional foods and dietary supplements. Moreover, the identified key active component, peptidoglycan, also shows potential for incorporation into topical skincare formulations targeting skin barrier dysfunction and inflammatory skin conditions. These findings provide theoretical support for the future development of postbiotic-based skin health products.

5. Conclusion

This study evaluated the role of *L. reuteri* CCFM1403 postbiotic in skin barrier repair and identified its key active components. Results indicate that the postbiotic CCFM1403 significantly ameliorates skin barrier impairment. This manifests in the restoration of HaCaT cell viability, upregulation of skin barrier functional proteins (FLG, LOR, IVL) and structural proteins (ZO-1, Occludin, Claudin), thereby promoting the recovery of both barrier structure and function. Animal study further confirmed that postbiotics administered orally via *L. reuteri* CCFM1403 effectively reduced TEWL, increased skin hydration levels, and improved skin tissue structure. Notably, the dead bacterial group demonstrated significantly superior repair effects compared to the live bacterial group, indicating that live bacteria are not a prerequisite for achieving these benefits.

Through hierarchical separation of the *L. reuteri* CCFM1403 bacterial structure, it was discovered that its primary active components are concentrated within the cell wall. Cell wall components significantly elevated the expression levels of barrier-related genes and proteins in both cells and animal models, demonstrating superior biological activity compared to protoplasts and surface proteins. Further research has revealed that peptidoglycan, the core structural component of the cell wall, is the key active substance responsible for this reparative effect. *L. reuteri* CCFM1403 peptidoglycan not only significantly promotes the expression of relevant barrier proteins, but also exhibits a distinct structural profile and stronger regulatory effects compared to other lactobacilli (*L. paracasei* and *L. plantarum*).

In summary, the *L. reuteri* CCFM1403 postbiotic demonstrates a significant role in restoring skin barrier function, with its unique peptidoglycan structure within the cell wall serving as the key material basis for this efficacy. This study not only elucidates the potential molecular by which postbiotics promote skin barrier repair but also provides theoretical foundations and novel directions for postbiotic-based skin health regulation and functional food development.

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7. Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The research described in this manuscript was conducted independently, and all experimental results and interpretations reflect the authors' genuine scientific findings.

1. Harris-Tryon TA, Grice EA. Microbiota and maintenance of skin barrier function, Science.376.6596(2022) 940. doi:10.1126/science.abo0693.
2. Zhang Q, Murawsky M, LaCount T, Kasting GB, Li SK. Transepidermal water loss and skin conductance as barrier integrity tests, Toxicol. In Vitro. 51 (2018). 129–135. doi:10.1016/j.tiv.2018.04.009.

3. Zhang LJ, Cao H, Li L, Zhao W, Zhang F. Oral and external intervention on the crosstalk between microbial barrier and skin via foodborne functional component, *J. Funct. Foods*. 92(2022)105075.doi:10.1016/j.jff.2022.105075.
4. Lee HJ, Kim M. Skin barrier function and the microbiome, *Int. J. Mol. Sci.* 23(21) (2022)13071. doi:10.3390/ijms232113071.
5. Gao T, Wang XY, Li YX, Ren FZ. The role of probiotics in skin health and related gut–skin axis: A review, *Nutrients*. 15.14 (2023)3123. doi:10.3390/nu15143123.
6. Aggarwal S, Sabharwal V, Kaushik P, Joshi A, Aayushi A, Suri M. Postbiotics: From emerging concept to application, *Front. Sustain. Food Syst.* 6(2022)887642. doi:10.3389/fsufs.2022.887642.
7. Coppola S, Avagliano C, Sacchi A, Laneri S, Calignano A, Voto L, et al. Potential clinical applications of the postbiotic butyrate in human skin diseases, *Molecules*. 27.6(2022)1849. doi:10.3390/molecules27061849.
8. Gholami A, Montazeri-Najafabady N, Ashoori Y, Kazemi K, Heidari R, Omidifar N, et al. The ameliorating effect of *Limosilactobacillus fermentum* and its supernatant postbiotic on cisplatin-induced chronic kidney disease in an animal model, *BMC Complement. Med. Ther.* 23.1(2023)243.doi:10.1186/s12906-023-04068-8.
9. Motei DE, Beteri B, Hepsomali P, Tzortzis G, Vulevic J, Costabile A. Supplementation with postbiotic from *Bifidobacterium breve* BB091109 improves inflammatory status and endocrine function in healthy females: A randomized, double-blind, placebo-controlled, parallel-groups study, *Front. Microbiol.* 14(2023)1273861. doi:10.3389/fmicb.2023.1273861.
10. Tian D, Han M. Bacterial peptidoglycan muropeptides benefit mitochondrial homeostasis and animal physiology by acting as ATP synthase agonists, *Dev. Cell.* 57.3(2022)361–369.doi:10.1016/j.devcel.2021.12.016.
11. Kim SJ, Chang J, Singh M. Peptidoglycan architecture of Gram-positive bacteria by solid-state NMR, *Biochim. Biophys. Acta Biomembr.* 1848.1(2015) 350–362.doi: 10.1016/j.bbamem.2014.05.031.
12. Sun QS, Liu XL, Li XL. Peptidoglycan-based immunomodulation, *Appl. Microbiol. Biotechnol.* 106.3(2022) 981–993.doi:10.1007/s00253-022-11795-4.
13. Fang ZF, Li LZ, Zhao JX, Zhang H, Lee YK, Lu WW, et al. *Bifidobacterium adolescentis* regulated immune responses and gut microbial composition to alleviate DNFB-induced atopic dermatitis in mice, *Eur. J. Nutr.* 59.7(2020) 3069–3081.doi: 10.1007/s00394-019-02145-8.
14. Jalali, Safura, et al. Functional properties and safety traits of *L. rhamnosus* and *L. reuteri* postbiotic extracts. *AMB Express* 14.1 (2024).doi: 114. 10.1186/s13568-024-01768-3.
15. Seo KH, Lee HG, Eor JY, Jeon HJ, Yokoyama W, Kim H. Effects of kefir lactic acid bacteria-derived postbiotic components on high-fat-diet-induced gut microbiota and obesity, *Food Res. Int.* 157(2022)111445.doi:10.1016/j.foodres.2022.111445.
16. Schwarzer M, Gautam UK, Makki K, Lambert A, Brabec T, Joly A, et al. Microbe-mediated intestinal NOD2 stimulation improves linear growth of undernourished infant mice, *Science*. 379.6635(2023)826–833.doi: 10.1126/science.ade9767.
17. Griffin ME, Tsukidate T, Hang HC. N-Arylpyrazole NOD2 agonists promote immune checkpoint inhibitor therapy, *ACS Chem. Biol.* 18.6(2023)1368–1377. doi:10.1021/acscchembio.3c00085.
18. Abd Allah FM, Elhosiny AM, Mohamed HF, Farrag AA, Elmeleigy MA. Enhanced antimicrobial activity of lactic acid bacteria through genome shuffling and genetic variability among shuffled strains, *World J. Microbiol. Biotechnol.* 39.5(2023)114.doi: 10.1007/s11274-023-03556-w.
19. Rajkumar J, Chandan N, Lio P, Shi V. The skin barrier and moisturization: Function, disruption, and mechanisms of repair, *Skin Pharmacol. Physiol.* 36.4(2023)174–185.doi:10.1159/000534136.
20. Bäsler K, Brandner JM. Tight junctions in skin inflammation, *Pflügers Arch. Eur. J. Physiol.* 469.1(2017) 3–14.doi:10.1007/s00424-016-1903-9.
21. Ma X, Pan Y, Zhao W, Sun PW, Zhao JF, Yan SY, et al. *Bifidobacterium infantis* strain YLGB-1496 possesses excellent antioxidant and skin barrier-enhancing efficacy in vitro, *Exp. Dermatol.* 31.7(2022)1089–1094.doi: 10.1111/exd.14583.
22. Lee, Y S, et al. "Anti-inflammatory and barrier-restoring effects of heat-killed *Lactiplantibacillus plantarum* postbiotics in an in vitro model of atopic dermatitis." *Journal of Applied Microbiology*, 136.10 (2025). doi: 10.1093/jambio/txaf256
23. Chang YC, Wang JMD, Chang HY, Zhou PH, Hahn RA, Gordon MK, et al. Expression of laminin γ 2 proteolytic fragments in murine skin following exposure to sulfur mustard, *Anat. Rec.* 303.6(2020)1642–1652. doi:10.1002/ar.24405.

24. Port H, Andelic M, Nyström A, Haack AM, Kalogeropoulos K, Nielsen VW, et al. Extracellular matrix alterations in inflammatory skin diseases: Emerging biomarkers and clinical implications, *Clin. Exp. Dermatol.* (2025)308.doi:10.1093/ced/llaf308
25. Pristavu MC, Diguta FC, Badea F, Aldea AC, Copaloni A, Matei F. *Pediococcus acidilactici* MI129-derived postbiotic formulations: In vitro insights into applications for health improvement, *Agrolife Sci. J.*14.1(2025)152–161.doi:10.17930/AGL2025116.
26. Wolf AJ. Peptidoglycan-induced modulation of metabolic and inflammatory responses, *Immunometabolism.*5.2(2023)e00024.doi:10.1097/IN9.00000000024.
27. Ferrand A, Al Nabhani Z, Tapias NS, et al. NOD2 expression in intestinal epithelial cells protects toward the development of inflammation and associated carcinogenesis. *Cell Mol Gastroenterol Hepatol.* 7.2(2019)357–69. doi: 10.1016/j.jcmgh.2018.10.009.
28. Ramanan D, Tang Mei S, Bowcutt R, et al. Bacterial sensor Nod2 prevents inflammation of the small intestine by restricting the expansion of the commensal bacteroides vulgatus. *Immunity.* 41.2(2014)311–24. doi: 10.1016/j.immuni.2014.06.015.