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Research Article

Evaluating Sialic Acid-Enhanced Skin Benefits of *Ligilactobacillus salivarius* (TCI153): A Randomized Double-Blind, Placebo-Controlled Trial

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ABSTRACT

With aging, skin elasticity decreases, and fine lines emerge. Recently, sialic acid has gained attention for its role in boosting skin hydration, elasticity, and radiance. The "gut-skin axis" concept also underscores how probiotics can enhance skin beauty by modulating the gut microbiome, reducing inflammation, and supporting healthier skin. This study aimed to evaluate the effects of the probiotic *Ligilactobacillus salivarius* (TCI153) on skin health through both clinical and *in vitro* approaches. In human dermal fibroblasts, TCI153 significantly increased sialic acid production (~280 μg/mL), upregulated *COL1A1* gene expression by 1.23-fold, enhanced elastin synthesis by 16%, and promoted collagen gel contraction by 5%, indicating potential benefits for skin firmness and extracellular matrix support. Clinically, sixty subjects were randomly divided into a TCI153 group and a placebo group, with each subject taking one sachet daily for eight consecutive weeks. Skin assessments, blood analyses, gut microbiota evaluations, and measurements of sialic acid and Epidermal Growth Factor (EGF) levels were conducted at baseline, week 4, and week 8. TCI153 significantly increased sialic acid and EGF levels, reduced melanin, improved skin lightness and elasticity, enhanced hydration, and decreased Transepidermal Water Loss (TEWL). Gut microbiota analysis also showed improved balance and a reduction in pathogenic bacteria. These results suggest that TCI153 supports skin health through both systemic and cellular pathways, highlighting its potential in beauty and wellness applications.

Keywords: *Ligilactobacillus salivarius*; Gut microbiota; Sialic acid; Skin

INTRODUCTION

Anti-aging is not solely about reducing wrinkles and preventing skin sagging but also fundamentally about enhancing overall skin health and beauty. With age, the skin loses collagen and elastin fibers, which leads to a reduction in firmness, elasticity, and the formation of fine lines. Key anti-aging ingredients, such as vitamins C and E, collagen, and polyphenols, have been shown to stimulate collagen production, support skin structure, and improve elasticity [1]. Additionally, these ingredients act as antioxidants that neutralize free radicals, thereby protecting cells from oxidative stress and delaying the skin aging process. The connection between probiotics and skin beauty is increasingly acknowledged, largely through the "gut-skin axis" concept [2].

Probiotics positively impact the microbiome balance in the gut, which can reduce systemic inflammation and toxin buildup that often manifests as skin issues like acne or eczema [2]. Specific probiotic strains enhance skin barrier functions by promoting hydration retention, which makes skin appear healthier and more radiant [3]. Moreover, probiotics can influence immune function, leading to improved skin resilience against environmental stressors [4]. These mechanisms collectively contribute to skin beauty by decreasing inflammatory skin conditions, improving moisture levels, and supporting overall skin health from within [5]. Consequently, the supplementation of probiotics has emerged as an effective strategy in modern skincare, allowing for a natural and holistic approach to achieving beautiful, radiant skin.

Ligilactobacillus salivarius, a well-researched probiotic, exhibits promising skin benefits through its impact on the gut microbiome and systemic health [6]. This probiotic strain aids in balancing the intestinal flora, which can decrease intestinal inflammation and, subsequently, systemic inflammation that may negatively affect the skin. *L. salivarius* is also noted for its antioxidant properties, which help to reduce free radicals that accelerate skin aging and damage

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[7]. By fortifying the skin barrier, this probiotic helps retain moisture and enhances skin texture, thereby promoting a healthy, youthful appearance [8]. Furthermore, the anti-inflammatory and immune-boosting effects of *L. salivarius* support clearer skin and a smoother complexion, demonstrating how this probiotic contributes to skin beauty through several interconnected mechanisms [9].

Sialic acid, also known as N-acetylneuraminic acid, is a valuable component found in bird's nest that is gaining attention for its skin-enhancing properties [10]. It acts as a potent hydrator, deeply moisturizing the skin and leaving it looking more supple and smooth. Additionally, sialic acid plays a role in stimulating collagen synthesis, which helps to improve skin elasticity and firmness [11]. The antioxidant nature of sialic acid also neutralizes free radicals, protecting the skin from UV-induced oxidative stress and slowing down the aging process [12]. By strengthening the skin barrier and improving its resilience, sialic acid supports long-term skin health and beauty. Consequently, regular supplementation or topical application of sialic acid offers a natural approach to maintaining youthful, radiant skin. Epidermal Growth Factor (EGF) significantly contributes to skin health and beauty [13]. EGF promotes skin cell regeneration, aids in tissue repair, and accelerates collagen synthesis, thereby enhancing skin elasticity and smoothness [14]. Additionally, EGF speeds up wound healing, reducing recovery time and supporting overall skin health [15]. It also helps to even skin tone by reducing pigmentation, resulting in a brighter complexion. Due to these properties, EGF is widely used in anti-aging and skin repair products, making it a key ingredient in maintaining youthful and healthy skin [16].

In this study, the *L. salivarius* TCI153 used was provided by TCI CO., Ltd. The purpose of this study was to evaluate the skin-enhancing efficacy of TCI153. *In vitro* experiments were performed using human dermal fibroblasts to examine TCI153's effects on biomarkers associated with skin structure and hydration, including sialic acid production, collagen biosynthesis, and elastin synthesis. In parallel, a total of sixty subjects were recruited and divided into two groups: The TCI153 group and the placebo group. Each subject consumed one sachet of powder daily for eight consecutive weeks. Skin assessments, blood samples, gut microbiota analysis, sialic acid levels, and human EGF analysis, as well as surveys, were conducted at baseline, after four weeks, and at the end of eight weeks.

MATERIALS AND METHODS

Probiotic product formulation

The probiotic strain used in this study, *L. salivarius* TCI153, was isolated from fresh breast milk. After initial culture on MRS plates supplemented with 0.5% cystine in an anaerobic environment at 37°C for 2 days, colonies were selected and streaked. A single colony was subjected to colony PCR for the amplification of the lactic acid bacteria 16S rRNA gene. The PCR products were analyzed and confirmed as *L. salivarius* through NCBI BLAST comparison. The strain was freeze-dried and formulated into a powder with a concentration of 1×10^{10} CFU/g. The supplement formulation included 150 mg of *L. salivarius* TCI153 (1.5×10^9 CFU/sachet), isomalt (hydrogenated palatinose), citric acid, blueberry flavour powder, and silicon dioxide. The placebo contained the same ingredients, excluding the probiotic strain. Both the probiotic and placebo products were identical in appearance, packaging, and taste to ensure blinding during the trial.

Cell culture

Human dermal fibroblasts (HDFs; ATCC® PCS-201-012) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin/streptomycin at 37°C in a humidified atmosphere containing 5% CO_2.

Sialic acid quantification

The ability of TCI153 to produce sialic acid was assessed under sterile culture conditions at 37°C for 24 hours. Sialic acid levels in the supernatant were measured using a colorimetric or fluorometric assay and quantified in μg/mL.

Collagen gel contraction assay

To evaluate skin-firming effects, a collagen lattice contraction assay was performed. Fibroblasts were embedded in a type I collagen matrix and allowed to polymerize at 37°C. After gel formation, the matrix was released, and samples were treated with TCI153. The gel diameter was measured after 24 hours to calculate the contraction rate.

Elastin biosynthesis assay

To measure elastin production, HDFs were treated with TCI153, and total elastin content was measured using an ELISA kit. Data were normalized to mock-treated control cells and expressed as a percentage of relative elastin production.

Collagen gene expression analysis

COL1A1 mRNA expression was measured using qPCR following treatment. RNA was extracted from fibroblasts, reverse-transcribed, and subjected to quantitative PCR using *COL1A1*-specific primers. Relative expression levels were normalized to GAPDH and calculated using the ΔΔCt method.

Clinical trial design

The study was registered in clinicaltrials.gov (No. NCT06166342), was performed under a protocol approved by the Antai Medical Care Cooperation Antai-Tian-Sheng memorial Hospital Institutional Review Board (Approval Number: TSMH-IRB23-091-B), and was conducted according to the code of ethics on human experimentation established by the Declaration of Helsinki (1964) and its amendments. Written informed consent was obtained from all participants after a full explanation of the study. A double-blinded, placebo controlled, randomized study was conducted. The subjects were randomly assigned to two groups with 30 subjects in each group (Table 1). The subjects were informed to consume one powder packet daily for 8 weeks. Before measurements, subjects were instructed to wash and wipe their face, and acclimatize for at least 30 min to the standardized laboratory conditions (room temperature 25°C, RH 55 ± 5%). This study is a double-blind, placebo-controlled trial. Subjects consumed one powder sachet daily for eight consecutive weeks, with assessments conducted at baseline, after 4 weeks, and after 8 weeks of consumption.

Table 1: The basic information of the subjects.

Group	Number of Female Subjects	Number of Male Subjects	Average Age (Years)
Placebo Group	27	3	49.7
TCI153 Group	24	6	47.9

Inclusion criteria for subjects: 1. Subjects aged over 18 years; 2. No severe systemic diseases, immunodeficiency, or autoimmune diseases; 3. No active allergic diseases; 4. No history of allergies to skincare or cosmetic products; 5. No recent use of corticosteroids or immunosuppressants.

Exclusion criteria for subjects: 1. Subjects who are not participating voluntarily; 2. Unwillingness to comply with study protocol requirements; 3. Diagnosed with skin disease, liver cirrhosis, or chronic renal failure by a physician; 4. Known allergies to cosmetics, medications, or food; 5. Pregnant or breastfeeding individuals; 6. Use of medications for chronic diseases; 7. Subjects who, within one month prior to testing, underwent laser facial treatment, chemical peels, or experienced prolonged sun exposure (more than 3 hours of direct sunlight exposure within one week); 8. Unwillingness to allow the publication of experimental result photographs; 9. Any other condition that, in the judgment of the staff, would make the subject unsuitable for the efficacy test.

Testing precautions: 1. During the study, subjects should maintain their pre-study dietary habits, lifestyle, and cosmetic use. Activities such as massages, skincare treatments at beauty clinics, or medical procedures are prohibited. 2. Subjects should minimize intense outdoor activities, such as sunbathing or vacationing, during the testing period. If outdoor activities are necessary, appropriate sun protection measures, such as wearing a hat, using an umbrella, wearing sun-protective clothing, and applying sunscreen, should be taken. 3. Consumption of the test product may cause gastrointestinal discomfort (e.g., abdominal pain or diarrhea). If any adverse reactions occur, subjects should discontinue use immediately and seek nearby medical assistance.

Clinical skin efficacy assessment

Skin assessments were conducted using various instruments. Melanin levels were measured on the upper cheek using a Soft Plus Skin Analyzer (Callegari 1930, Italy) with dual-wavelength measurements at 505 nm (green light) and 875 nm (infrared). Skin tone was evaluated with the Chroma Meter MM500 (Minolta, Japan), utilizing the CIE color system to obtain L^* values, where higher values indicate greater brightness. Skin hydration was assessed *via* the Corneometer CM825 (C+K, Germany), which employs a capacitance method to determine moisture content. TEWL was measured on the upper cheek with a Tewameter TM300 (CK, Germany) to evaluate the integrity of the stratum corneum and skin barrier function. Skin elasticity was determined using the Cutometer MPA580 (C+K, Germany), applying negative pressure to measure skin's viscoelastic properties. Full-face skin texture analysis was conducted with the VISIA Complexion Analysis System (Canfield, USA), which captures high-resolution images in a controlled lighting environment and analyzes skin smoothness by detecting shadow variations in standard white light. Additionally, a self-assessment questionnaire was administered to subjects to gather data on basic conditions, skin health, bowel movement frequency, stool characteristics, and defecation difficulty.

Human EGF analysis

Using the Human EGF ELISA Kit (MBS2709586), follow the protocol as outlined in the manual. Begin by adding 100 µL of standard or sample to each well and incubate at 37°C for 1 hour. After incubation, aspirate the liquid from each well and add 100 µL of the prepared detection reagent A. Incubate again at 37°C for 1 hour. Following this step, aspirate and wash the wells three times. Add 100 µL of the prepared detection reagent B and incubate at 37°C for 30 minutes. After incubation, aspirate the liquid and wash the wells five times. Then, add 90 µL of substrate solution and incubate at 37°C for 10 to 20 minutes. Finally, add 50 µL of stop solution and immediately read the results at 450 nm.

Human sialic acid analysis

According to the Sialic Acid ELISA Kit (MBS163087) manual, add the sample and ELISA reagent to each well and incubate at 37°C for 1 hour. Following incubation, wash the plate five times. Next, add substrate solutions A and B, then incubate at 37°C for 10 minutes. Stop the reaction by adding the stop solution, allowing color to develop. Finally, measure the OD value within 10 minutes for precise results.

Fecal sample collection and gut microbiome analysis

Subjects collected fecal samples at home following a validated protocol provided by BIOTOOLS Co., LTD. Samples were aliquoted into 1.5 mL eppendorf tubes and stored at -80°C until further laboratory analysis. Total genomic DNA was extracted from the samples using a column-based method, such as the QIAamp PowerFecal DNA Kit (Qiagen). DNA concentration was quantified and adjusted to 5 ng/µL for subsequent processing. For 16S rRNA gene sequencing, the V3-V4 region was amplified using a specific primer set (F: 5′-CCTACGGGNGGCWGCAG-3′, R: 5′-GACTACHVGGGTATCTAATCC-3′) according to the 16S Metagenomic Sequencing Library Preparation protocol (Illumina). In brief, 12.5 ng of gDNA was used for PCR with KAPA HiFi HotStart ReadyMix (Roche), under the following conditions: initial denaturation at 95°C for 3 minutes; 25 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds; followed by a final extension at 72°C for 5 minutes, with a hold at 4°C. PCR products were visualized on a 1.5% agarose gel, and samples showing a prominent band around 500 bp were selected and purified using AMPure XP beads for library preparation. The sequencing library was prepared according to the 16S Metagenomic Sequencing Library Preparation protocol (Illumina). A secondary PCR was performed using the 16S rRNA V3-V4 region PCR amplicon and the Nextera XT Index Kit, which included dual indices and Illumina sequencing adapters. Quality assessment of the indexed PCR products was conducted with the Qubit 4.0 Fluorometer (Thermo Scientific) and the Qsep100TM system. Equal amounts of indexed PCR products were pooled to generate the final sequencing library, which was subsequently sequenced on an Illumina MiSeq platform, yielding paired 300-bp reads.

Statistical analysis

The comparison of measurement results for skin parameters among groups and between groups was analyzed by student's t-test through GraphPad Prism, as $p<0.05$ was considered statistical significance.

RESULTS

TCI153 enhanced extracellular matrix remodeling and sialic acid *in vitro*

In this study, TCI153 was shown to significantly enhance multiple aspects of extracellular matrix activity in human dermal fibroblasts. After 24 hours of incubation under sterile conditions at 37 °C, TCI153 produced the highest level of sialic acid (~280 μg/mL), outperforming comparator strains LH49: *Streptococcus thermophilus*, LH37: *Lacticaseibacillus rhamnosus*, L208: *Bifidobacterium breve*. In a collagen gel contraction assay (Figure 1A), TCI153 treatment led to a 5% reduction in gel size compared to controls, indicating increased fibroblast-mediated matrix contraction and skin firming potential (Figure 1B). Additionally, elastin biosynthesis was significantly upregulated by 16% in the TCI153-treated group relative to the mock control, suggesting improved skin elasticity (Figure 1C). Quantitative PCR analysis further revealed that TCI153 increased *COL1A1* gene expression to 1.23-fold, supporting its role in promoting collagen biosynthesis (Figure 1D). Collectively, these findings demonstrated that TCI153 effectively promoted skin firmness and rejuvenation-related biomolecular activities *in vitro*.

Increase in sialic acid and EGF levels following TCI153 supplementation

Firstly, this study aimed to investigate whether *L. salivarius* TCI153 increased the levels of sialic acid and EGF in the body. The results showed that the TCI153 probiotic group significantly increased blood sialic acid levels after 8 weeks. Subjects in the TCI153 group showed an increase in blood sialic acid concentration from a baseline of 56.3 mg/dL to 116.3 mg/dL, with a 100% improvement rate, while the placebo group showed no significant change. Additionally, the sialic acid level change in the TCI153 group increased from 0.00 mg/dL at week 0 to 59.92 mg/dL at week 8, which was 102% higher than the change observed in the placebo group (2.54 mg/dL) (Figure 2A and 2B). The results showed that the TCI153 probiotic group significantly increased blood Epidermal Growth Factor (EGF) levels after 8 weeks. Subjects in the TCI153 group exhibited an increase in EGF concentration from a baseline of 161.7 pg/mL to 188.6 pg/mL, representing a 16.6% increase, with an improvement rate of 83.3%. Additionally, the change in EGF levels in the TCI153 group rose from 0.00 pg/mL at week 0 to 26.85 pg/mL at week 8, which was 26.0% higher than the change observed in the placebo group (-17.46 pg/mL) (Figure 2C and 2D). Additionally, Table 2 showed that taking TCI153 did not affect blood lipids, liver, or kidney function. These findings demonstrated that TCI153 probiotics significantly elevated sialic acid and EGF in the body.

TCI153 had the potential to reduce melanin and enhance skin lightness

Next, this study investigated whether *L. salivarius* TCI153 could reduce skin hyperpigmentation. The results showed that after taking TCI153 probiotics for 8 weeks, subjects experienced a significant 4.3% reduction in their melanin index compared to baseline (the start of the trial). The improvement rate reached 73.3%, indicating that most subjects showed noticeable improvement in reducing skin pigmentation. Additionally, the change in melanin levels among subjects showed a significant 4.9% reduction compared to the placebo group (Figure 3A and 3B). The results indicated that after 8 weeks of TCI153 probiotic supplementation, subjects experienced a significant 2.0% improvement in skin lightness compared to baseline, with an 80% improvement rate, suggesting that most subjects showed noticeable enhancement in skin brightness. Compared to the placebo group, the change in skin lightness for the TCI153 group was 1.8% higher (Figure 3C-3E).

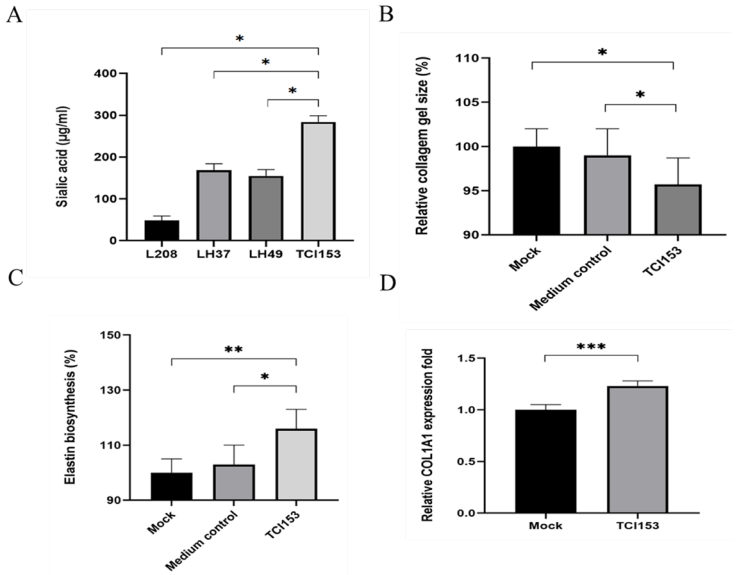

Figure 1: *In vitro* assessment of the effects of TCI153 on skin-related biomarkers in HDFs. (A) Sialic acid levels in culture supernatants were measured after 24-hour incubation at 37°C with different probiotic strains under sterile conditions. LH49: *Streptococcus thermophilus*, LH37: *Lacticaseibacillus rhamnosus*, L208: *Bifidobacterium breve*. (B) Collagen gel contraction was assessed in HDFs embedded in type I collagen matrices; relative gel size was measured after 24 hours. (C) Elastin biosynthesis in HDFs treated with TCI153 or controls was quantified using Enzyme-Linked Immunosorbent Assay (ELISA). (D) Expression of the collagen type I alpha 1 (*COL1A1*) gene was analyzed by quantitative real-time PCR (qPCR) in HDFs following treatment. **Note:** *, p<0.05, **, p<0.01, ***, p<0.001.

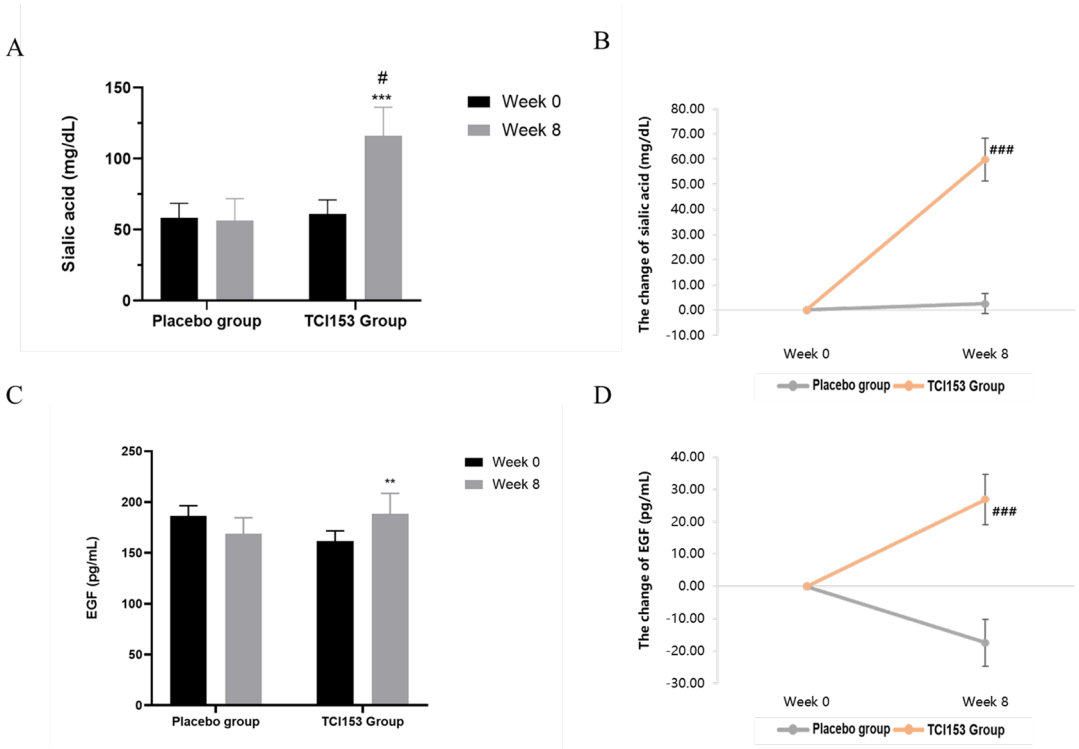

Figure 2: The supplementation of TCI153 can increase the levels of sialic acid and EGF. Sixty subjects were divided into two groups: placebo group and TCI153 group. The subjects received one sachet daily for eight consecutive weeks. Afterward, blood samples were analyzed for (A) sialic acid, (B) the change in sialic acid, (C) EGF, and (D) the change in EGF. *, compared with baseline (week 0) (**Note:** *, p<0.05, **, p<0.01, ***, p<0.001). #, compared with placebo group (#, p<0.05, ##, p<0.01, ###, p<0.001).

Table 2: Average values of blood biochemistry analysis of subjects at 0 and 8 weeks after using the product.

Blood Biochemistry Analysis	TCI153 Group		Placebo Group	
Weeks	0	8	0	8
Total Cholesterol	193.9	184.3*	198.1	201
Triglycerides	153.3	143.2	138.3	143.4
sGOT	17.7	15.8*, #	17.5	17.9
sGPT	16.1	15	16.1	16.9
Blood Urea Nitrogen	14.6	14.7	15	14.1
Creatinine	0.71	0.72	0.7	0.73*
Blood Glucose AC	91.7	92.3	89.2	85.7*

Note: sGOT: Serum Glutamate Oxaloacetate Transaminase; sGPT: Serum Glutamate Pyruvate Transaminase

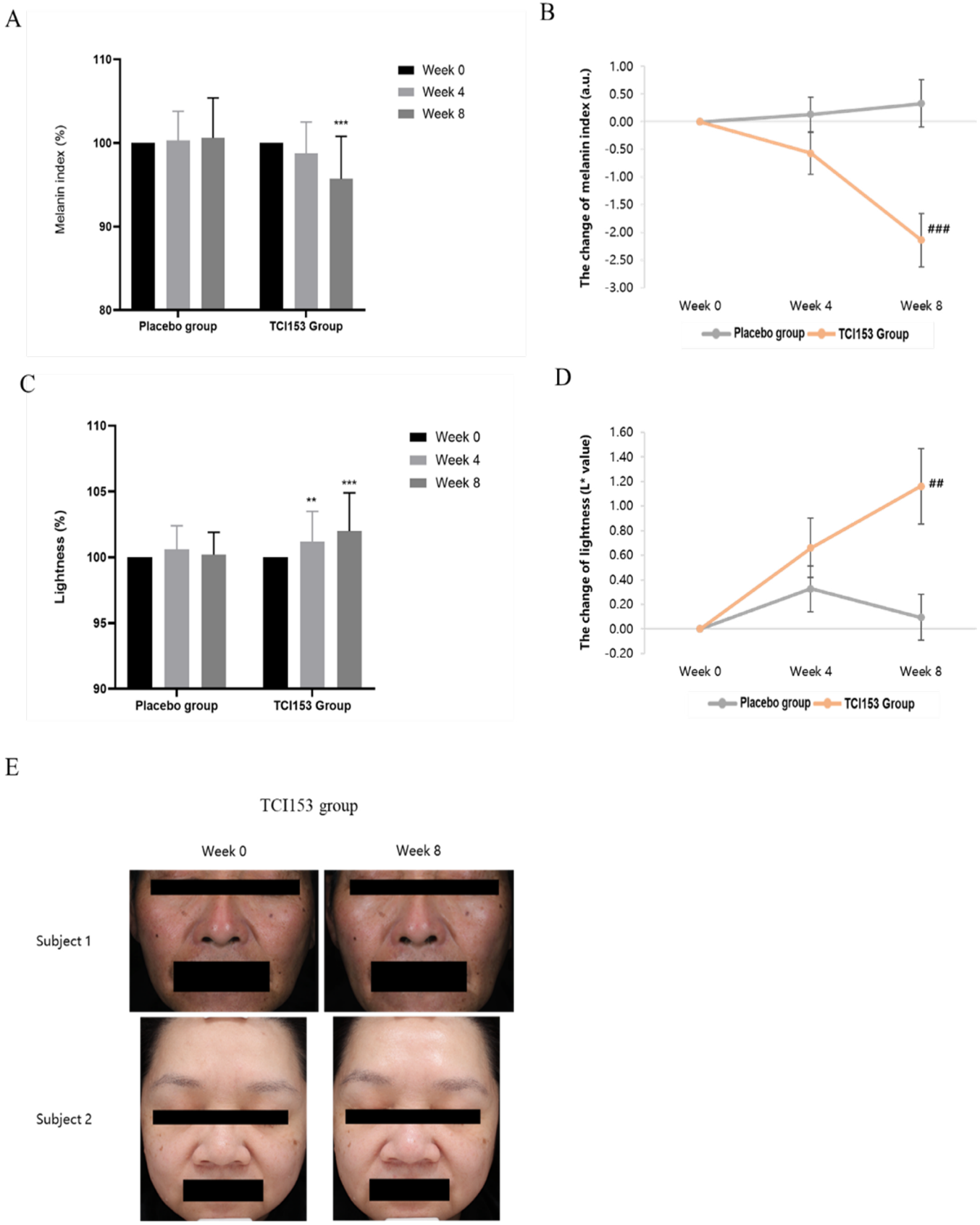

Figure 3: The supplementation of TCI153 can reduce melanin levels and enhance lightness. Sixty subjects were divided into two groups: placebo group and TCI153 group. Subjects took one sachet daily for eight consecutive weeks, followed by a skin assessment. (A) Melanin index, (B) the change in melanin, (C) lightness, (D) the change in lightness, (E) photos of subjects' lightness. **Note:** *, compared with baseline (week 0) (*, $p<0.05$, **, $p<0.01$, ***, $p<0.001$). #, compared with placebo group (#, $p<0.05$, ##, $p<0.01$, ###, $p<0.001$).

TCI153 had the potential to enhance skin elasticity and reduce texture

The results indicated that after 8 weeks of TCI153 probiotic supplementation, subjects had a significant 4.8% improvement in skin elasticity compared to baseline, with an improvement rate of 86.7%, suggesting that most subjects showed noticeable enhancement in skin elasticity. Compared to the placebo group, the TCI153 group demonstrated a significant 4.8% increase in elasticity change (Figure 4A and 4B). The results indicated that after 8 weeks of TCI153 probiotic supplementation, subjects experienced a significant 6.8% reduction in skin roughness compared to baseline, with an improvement rate of 66.7%, suggesting that most subjects showed noticeable improvement in skin texture. Additionally, compared to the placebo group, the TCI153 group demonstrated a significant 16.3% reduction in texture change (Figure 4C and 4E).

TCI153 can enhance skin hydration and reduce TEWL

The results indicated that after 8 weeks of TCI153 probiotic supplementation, skin hydration significantly increased by 3.8% compared to baseline, with an improvement rate of 70% among subjects. The change in hydration showed a marked increase of 4.9% compared to the placebo group. After 8 weeks of TCI153 probiotic supplementation, TEWL was significantly reduced by 3.2% compared to baseline, with an improvement rate of 76.7% among participants. The change in TEWL demonstrated a significant reduction of 4.8% compared to the placebo group (Figure 5A and 5B). Results from the subjective questionnaire indicated that after 8 weeks of TCI153 probiotic supplementation, over 60% of subjects perceived an improvement in skin tone dullness and skin radiance. Additionally, 70% of subjects reported improved bowel regularity, and 76.7% expressed satisfaction with the overall effects of the product (Figure 5C).

TCI153 probiotic regulated gut balance and reduced pathogens

Gut microbiota analysis indicated that after 8 weeks of TCI153 probiotic supplementation, the overall balance of the native microbiota remained unchanged (Figure 6A). Additionally, subjects maintained gut microbial richness (Figure 6B), with a reduction in the relative abundance of pathogenic bacteria (Figure 6C).

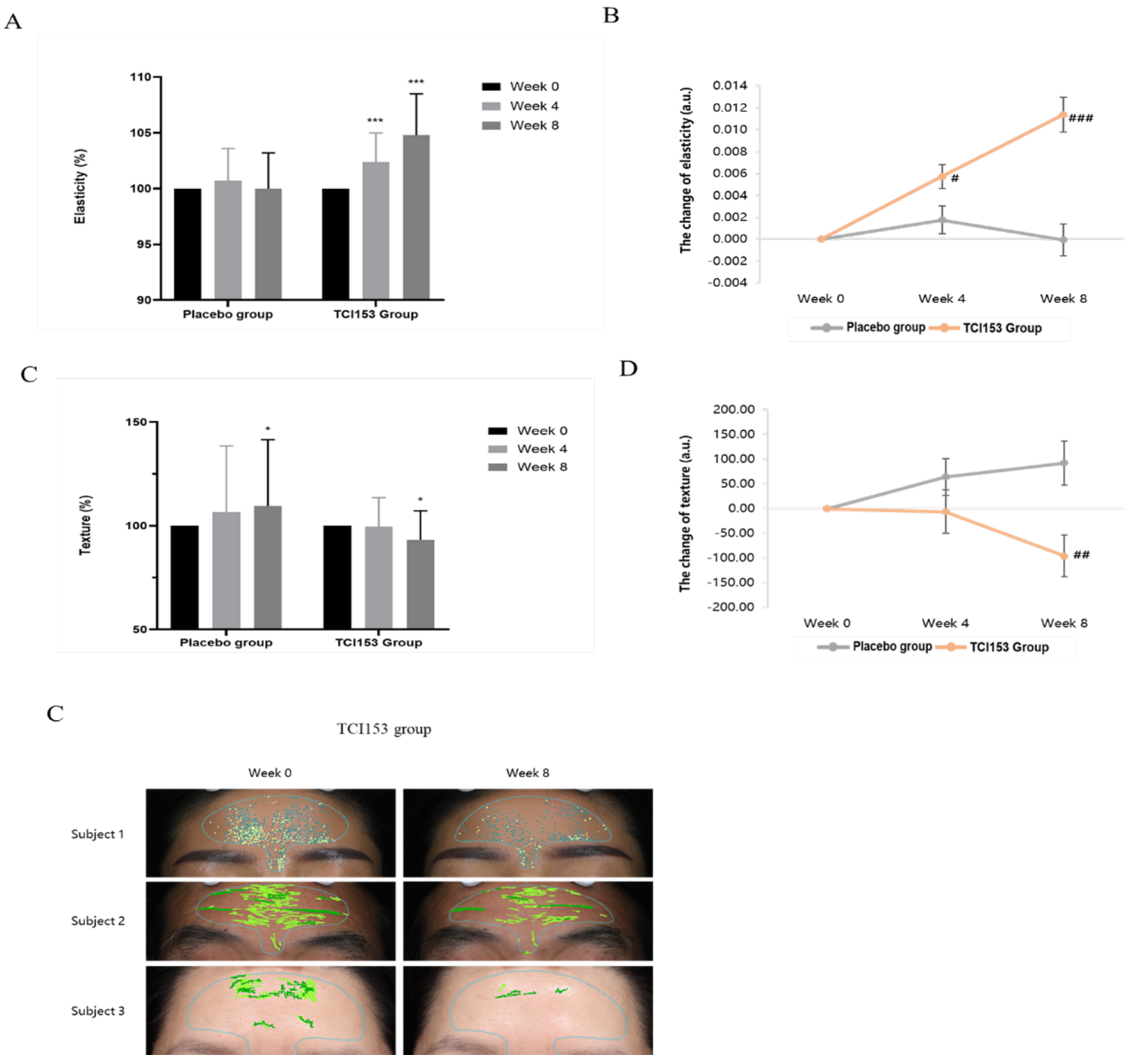

Figure 4: The supplementation of TCI153 can enhance elasticity and reduce texture. Sixty subjects were divided into two groups: placebo group and TCI153 group. Subjects took one sachet daily for eight consecutive weeks, followed by a skin assessment. (A) Elasticity, (B) the change in elasticity, (C) texture, (D) the change in texture, (E) photos of subjects' texture. **Note:** *, compared with baseline (week 0) (*, p<0.05, **, p<0.01, ***, p<0.001). #, compared with placebo group (#, p<0.05, ##, p<0.01, ###, p<0.001).

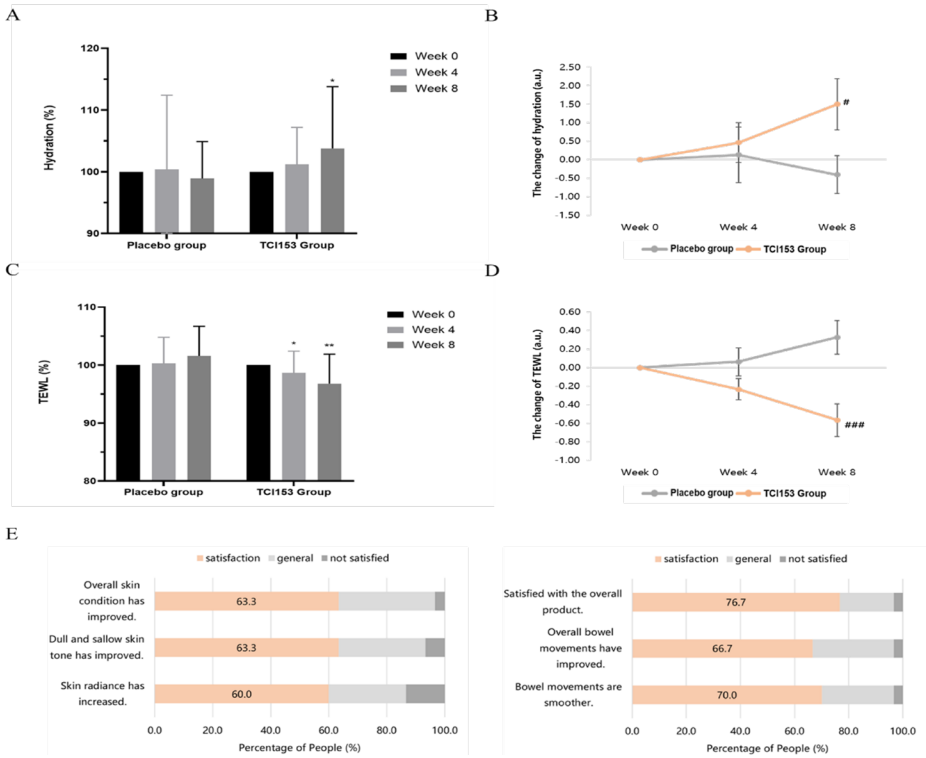

Figure 5: The supplementation of TCI153 can increase hydration and reduce TEWL. Sixty subjects were divided into two groups: placebo group and TCI153 group. Subjects took one sachet daily for eight consecutive weeks, followed by a skin assessment. (A) Hydration, (B) the change in hydration, (C) TEWL, (D) the change in TEWL. (E) Sensory perception questionnaire. **Note:** *, compared with baseline (week 0) (*, p<0.05, **, p<0.01, ***, p<0.001). #, compared with placebo group (#, p<0.05, ##, p<0.01, ###, p<0.001).

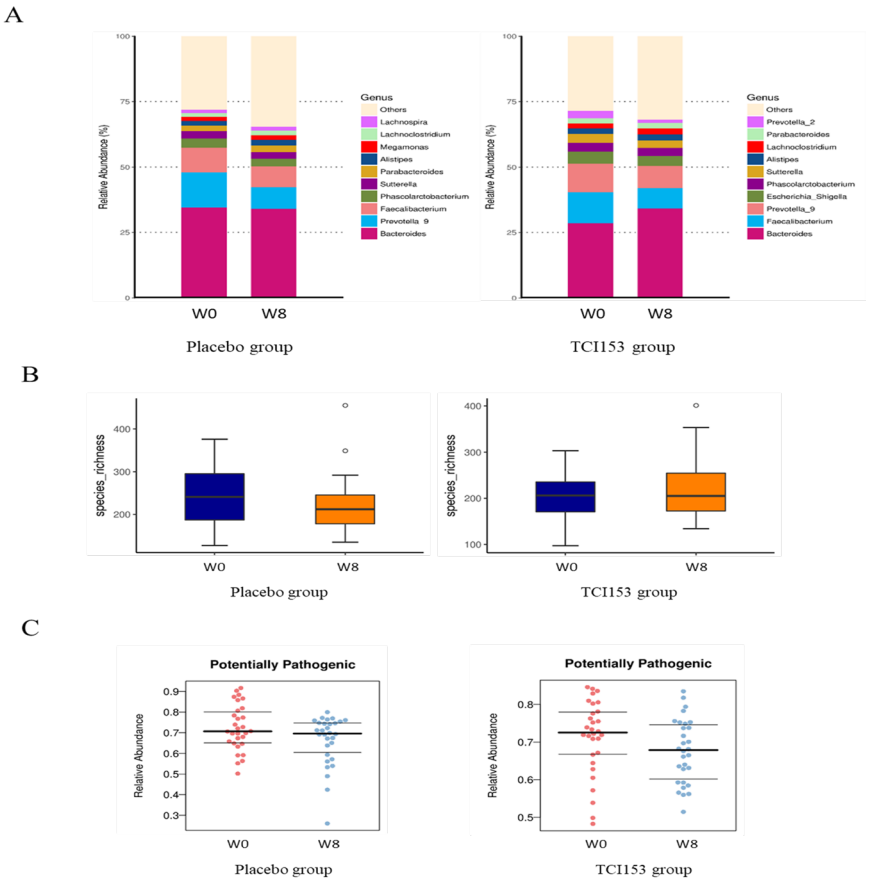

Figure 6: The supplementation of TCI153 can regulate gut balance and reduce pathogens. Subjects were asked to take placebo or TCI153 for 8 weeks and stool was collected to examine (A and B) gut microbiome by NGS, (C) the abundance of intestinal pathogenic flora.

DISCUSSION

This study demonstrated that *L. salivarius* TCI153 promoted sialic acid production (~280 μg/mL), increased elastin by 16%, enhanced collagen gel contraction by 5%, and upregulated *COL1A1* gene expression by 1.23-fold *in vitro*. These cellular findings align with the clinical outcomes, indicating that after 8 weeks of *L. salivarius* TCI153 supplementation, sialic acid and EGF levels were increased. In terms of skin health benefits, TCI153 significantly reduced melanin, enhanced lightness, improved elasticity, and reduced texture, while increasing skin hydration and reducing TEWL. Additionally, TCI153 maintained gut microbiota balance and richness, significantly lowering the relative abundance of pathogenic bacteria, demonstrating multiple positive effects on skin and gut health. *L. salivarius* is known for its probiotic benefits, particularly in modulating gut health and supporting the immune system. Sialic acid, often associated with gut health, plays a critical role in immune regulation and maintaining the gut's mucosal barrier [17]. Some studies suggest that sialic acid aids in modulating the gut microbiota by promoting beneficial bacteria and reducing pathogenic species [18]. Furthermore, sialic acid can act as a signaling molecule, supporting immune functions by binding to Siglec receptors on immune cells, which helps to distinguish self from non-self, reducing unnecessary inflammation [19]. Additionally, sialic acid's presence in the gut is essential for gut health due to its anti-inflammatory properties [17]. *L. salivarius* can enhance the availability of sialic acid in the gut, promoting an environment conducive to maintaining a balanced microbiota, which may indirectly improve skin health by reducing systemic inflammation [6]. While direct interactions between *L. salivarius* and EGF are less commonly discussed, there is evidence that probiotics like *L. salivarius* can promote EGF-related benefits indirectly by reducing systemic inflammation and improving gut health, which in turn may support EGF pathways involved in tissue repair and skin health [20]. EGF is crucial for cell proliferation and regeneration, particularly through the activation of the EGF receptor (EGFR) [21]. A study highlighted that sialylation on cell surfaces can facilitate EGF signaling, indirectly suggesting that a healthy gut, supported by *L. salivarius*, can create favorable conditions for EGFR activation [22]. Furthermore, the anti-inflammatory effects of *L. salivarius* may boost EGF's effectiveness in promoting collagen synthesis and wound healing by reducing pro-inflammatory cytokines, which could otherwise hinder skin regeneration [23]. These findings collectively suggest that *L. salivarius* may support EGF activity through enhanced microbiota balance and a reduction in systemic inflammatory responses, both of which are crucial for maintaining healthy skin and promoting cell turnover. Consistent with our results, *L. salivarius* TCI153 can increase EGF and sialic acid.

L. salivarius has shown potential in modulating the gut-skin axis, which can indirectly influence melanin production [24]. By improving gut health and reducing inflammation, it may help lower systemic oxidative stress, a factor that can contribute to increased melanin synthesis and hyperpigmentation [25]. Reduced inflammation may decrease melanocyte-stimulating factors, potentially leading to lighter, more even-toned skin [26]. Additionally, studies have shown that probiotics can contribute to an increase in antioxidant enzymes, which may reduce melanin synthesis by protecting skin cells from oxidative damage that triggers hyperpigmentation [4]. *L. salivarius* can influence skin elasticity by promoting gut health and reducing inflammatory markers that may impair collagen synthesis [4]. Enhanced gut microbiota balance has been linked to increased production of Short-Chain Fatty Acids (SCFAs), which can have systemic benefits, including supporting the production of collagen and elastin in the skin [27]. Collagen and elastin are vital for maintaining skin firmness and texture. Furthermore, by reducing oxidative stress, *L. salivarius* may help in reducing glycation—a process that can lead to stiffer collagen and thus worsened skin texture. *L. salivarius* may improve skin hydration by supporting the production of hyaluronic acid and ceramides through the gut-skin axis [28]. Hyaluronic acid helps retain moisture in the skin, while ceramides strengthen the skin barrier, both of which are essential for maintaining hydration [29]. By enhancing the skin's barrier function, *L. salivarius* may also reduce TEWL, which is a measure of how much water evaporates from the skin's surface [30]. A strong skin barrier prevents excessive water loss, ensuring better hydration levels and reducing dryness [31]. Consistent with our findings, *L. salivarius* TCI153 can effectively improve melanin reduction, skin lightness, elasticity, texture, hydration, and TEWL.

L. salivarius TCI153 supports gut balance by promoting a favorable environment for beneficial bacteria. This probiotic is known to produce metabolites like lactic acid and SCFAs, which lower the gut pH, making it less hospitable for harmful bacteria [32]. The acidic environment helps beneficial bacteria thrive while inhibiting pathogenic bacteria that prefer more neutral pH conditions [33]. Moreover, *L. salivarius* has been shown to improve the composition of the gut microbiota by reinforcing the abundance of commensal bacteria, which play a key role in maintaining microbial diversity and ecosystem stability [6]. This probiotic can outcompete pathogens for nutrients and adhesion sites in the gut, thereby preventing harmful bacteria from proliferating and disrupting the microbial balance. *L. salivarius* produces bacteriocins, which are antimicrobial peptides that specifically target and inhibit pathogenic bacteria without affecting beneficial bacteria. These bacteriocins can directly kill or inhibit the growth of pathogens like *Clostridium* and *Salmonella*, thus helping to reduce the overall pathogenic load in the gut [34]. Additionally, by promoting beneficial microbial populations, *L. salivarius* enhances the gut's natural defense mechanisms, making it harder for pathogens to colonize and thrive [35]. By balancing gut microbiota and reducing harmful bacteria, *L. salivarius* contributes to overall gut health, reducing inflammation, and potentially impacting systemic health, including skin health, through the gut-skin axis [2].

CONCLUSION

L. salivarius TCI153 supplementation over 8 weeks demonstrated significant benefits in enhancing skin health, with notable increases in sialic acid and EGF levels, reductions in melanin, and improvements in skin lightness, elasticity, texture, hydration, and TEWL. Additionally, TCI153 maintained gut microbiota balance and reduced pathogenic bacteria, suggesting positive impacts on overall gut health. These findings indicate that *L. salivarius* TCI153 may be a valuable probiotic for skincare and gut health, with potential applications in beauty and wellness products aimed at skin hydration, anti-aging, and microbiota support. Future research could explore its long-term benefits and its role in specific skincare formulations to maximize its therapeutic effects.

AUTHOR CONTRIBUTIONS

Chia-Hua Liang, Chi-Fu Chiang: Data curation, Investigation, Methodology, Writing-original draft. Chia-Hua Liang:

Formal analysis, Software, Writing-review and editing. Yung-Hsiang Lin: Resources. Chia-Hua Liang: Conceptualization, Funding acquisition, Project administration, Supervision, Writing-review and editing. Shu-Ting Chan, Yung-Kai Lin, Chi-Fu Chiang: Conceptualization, Funding acquisition, Project administration, Writing-review and editing.

REFERENCES

1. He X, Gao X, Guo Y, Xie W. Research progress on bioactive factors against skin aging. Int J Mol Sci. 2024;25(7):3797.
2. Mahmud MR, Akter S, Tamanna SK, Mazumder L, Esti IZ, Banerjee S, et al. Impact of gut microbiome on skin health: Gut-skin axis observed through the lenses of therapeutics and skin diseases. Gut Microbes. 2022;14(1):2096995.
3. Dou J, Feng N, Guo F, Chen Z, Liang J, Wang T, et al. Applications of probiotic constituents in cosmetics. Molecules. 2023;28(19):6765.
4. Gao T, Wang X, Li Y, Ren F. The role of probiotics in skin health and related gut-skin axis: A review. Nutrients. 2023;15(14):3123.
5. Lee HJ, Kim M. Skin barrier function and the microbiome. Int J Mol Sci. 2022;23(21):13071.
6. Yang Y, Song X, Wang G, Xia Y, Xiong Z, Ai L. Understanding *Ligilactobacillus salivarius* from probiotic properties to omics technology: A review. Foods. 2024;13(6):895.
7. Fernandes A, Rodrigues PM, Pintado M, Tavaria FK. A systematic review of natural products for skin applications: Targeting inflammation, wound healing, and photo-aging. Phytomedicine. 2023;115:154824.
8. Drago L, Iemoli E, Rodighiero V, Nicola L, De Vecchi E, Piconi S. Effects of Lactobacillus salivarius LS01 (DSM 22775) treatment on adult atopic dermatitis: A randomized placebo-controlled study. Int J Immunopathol Pharmacol. 2011;24(4):1037-48.
9. Saravanan P, Pooja R, Balachander N, Sing KKR, Silpa S, Rupachandra S. Anti-inflammatory and wound healing properties of lactic acid bacteria and its peptides. Folia Microbiologica. 2023;68(3):337-53.
10. Rashed AA, Ahmad H, Abdul Khalid SK, Rathi DN. The potential use of sialic acid from edible bird's nest to attenuate mitochondrial dysfunction by *in vitro* study. Front Pharmacol. 2021;12:633303.
11. Zhu W, Zhou Y, Guo L, Feng S. Biological function of sialic acid and sialylation in human health and disease. Cell Death Discovery. 2024;10(1):415.
12. Poljšak B, Dahmane R. Free radicals and extrinsic skin aging. Dermatol Res Pract. 2012;2012(1):135206.
13. Shin SH, Koh YG, Lee WG, Seok J, Park KY. The use of epidermal growth factor in dermatological practice. Int Wound J. 2023;20(6):2414-23.
14. Xia Y, You XE, Chen H, Yan YJ, He YC, Ding SZ. Epidermal growth factor promotes mesenchymal stem cell-mediated wound healing and hair follicle regeneration. Int J Clin Exp Pathol. 2017;10(7):7390.
15. Yamakawa S, Hayashida K. Advances in surgical applications of growth factors for wound healing. Burns Trauma. 2019;7.
16. Ganceviciene R, Liakou AI, Theodoridis A, Makrantonaki E, Zouboulis CC. Skin anti-aging strategies. Dermatoendocrinol. 2012;4(3):308-19.
17. Bell A, Severi E, Owen CD, Latousakis D, Juge N. Biochemical and structural basis of sialic acid utilization by gut microbes. J Biol Chem. 2023;299(3):102989.
18. Jennings MP, Day CJ, Atack JM. How bacteria utilize sialic acid during interactions with the host: Snip, snatch, dispatch, match and attach. Microbiol. 2022;168(3):001157.
19. Gianchecchi E, Arena A, Fierabracci A. Sialic acid-siglec axis in human immune regulation, involvement in autoimmunity and cancer and potential therapeutic treatments. Int J Mol Sci. 2021;22(11):5774.
20. Huang R, Wu F, Zhou Q, Wei W, Yue J, Xiao B, Luo Z. *Lactobacillus* and intestinal diseases: Mechanisms of action and clinical applications. Microbiol Res. 2022;260:127019.
21. Wee P, Wang Z. Epidermal growth factor receptor cell proliferation signaling pathways. Cancers. 2017;17;9(5):52.
22. Ankenbauer KE, Rao TC, Mattheyses AL, Bellis SL. Sialylation of EGFR by ST6GAL1 induces receptor activation and modulates trafficking dynamics. J Biol Chem. 2023;299(10).
23. Xiao T, Yan Z, Xiao S, Xia Y. Proinflammatory cytokines regulate epidermal stem cells in wound epithelialization. Stem Cell Res Ther. 2020;11(1):232.
24. Yang J, Wang J, Liu Z, Chen J, Jiang J, Zhao M, et al. *Ligilactobacillus salivarius* improve body growth and anti-oxidation capacity of broiler chickens *via* regulation of the microbiota-gut-brain axis. BMC Microbiol. 2023;23(1):395.
25. Yang X, Tang C, Zhao Q, Jia Y, Qin Y, Zhang J. Melanin: A promising source of functional food ingredient. J Funct Foods. 2023;105:105574.
26. Fu C, Chen J, Lu J, Yi L, Tong X, Kang L, et al. Roles of inflammation factors in melanogenesis. Mol Med Rep. 2020;21(3):1421-30.
27. Portincasa P, Bonfrate L, Vacca M, De Angelis M, Farella I, Lanza E, et al. Gut microbiota and short chain fatty acids: implications in glucose homeostasis. Int J Mol Sci. 2022;23(3):1105.
28. Zgutka K, Tkacz M, Tomasiak P, Tarnowski M. A role for advanced glycation end products in molecular ageing. Int J Mol Sci. 2023;24(12):9881.
29. Bravo B, Correia P, Gonçalves Junior JE, Sant'Anna B, Kerob D. Benefits of topical hyaluronic acid for skin quality and signs of skin aging: From literature review to clinical evidence. Dermatol Ther. 2022;35(12):e15903.
30. Wang DQ, Li X, Zhang RY, Yuan C, Yan B, Humbert P, et al. Effects of investigational moisturizers on the skin barrier and microbiome following exposure to environmental aggressors: A randomized clinical trial and ex vivo analysis. J Clin Med. 2023;12(18):6078.
31. Kono T, Miyachi Y, Kawashima M. Clinical significance of the water retention and barrier function-improving capabilities of ceramide-containing formulations: A qualitative review. J Dermatol. 2021;48(12):1807-16.
32. Chandrasekaran P, Weiskirchen S, Weiskirchen R. Effects of probiotics on gut microbiota: An overview. Int J Mol Sci. 2024;25(11):6022.
33. Icer MA, Özbay S, Ağagündüz D, Kelle B, Bartkiene E, Rocha JM, et al. The impacts of acidophilic lactic acid bacteria on food and human health: A review of the current knowledge. Foods. 2023;12(15):2965.
34. Anjana A, Tiwari SK. Bacteriocin-producing probiotic lactic acid bacteria in controlling dysbiosis of the gut microbiota. Front Cell Infect Microbiol. 2022;12:851140.
35. Moturi J, Kim KY, Hosseindoust A, Lee JH, Xuan B, Park J, et al. Effects of *Lactobacillus salivarius* isolated from feces of fast-growing pigs on intestinal microbiota and morphology of suckling piglets. Sci Rep. 2021;11(1):6757.