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Streptococcus thermophilus-Derived Cell-Free Lysate Reduces Melanogenesis in Human Melanocytes Through Sphingomyelinase-Dependent Signaling

Serena Altamura [1,†], Alessia Ciafarone [1,†], Zoran Evtoski [2], Maria Grazia Cifone [3], Maurizio Giuliani [3], Francesca Lombardi [3,*] and Benedetta Cinque [3,*]

[1] Department of Physical and Chemical Sciences, University of L'Aquila, Coppito, 67100 L'Aquila, Italy; serena.altamura@univaq.it (S.A.); alessia.ciafarone@univaq.it (A.C.)
[2] 3CSIC–Instituto de Agroquímica y Tecnología de Alimentos (IATA), 46980 Paterna, Spain; zoran.evtoski@iata.csic.es
[3] Department of Life, Health & Environmental Sciences, University of L'Aquila, Coppito, 67100 L'Aquila, Italy; mariagrazia.cifone@univaq.it (M.G.C.); maurizio.giuliani@univaq.it (M.G.)
* Correspondence: francesca.lombardi@univaq.it (F.L.); benedetta.cinque@univaq.it (B.C.)
† These authors contributed equally to this work.

Abstract

Background: Bioactive products derived from probiotic microorganisms are increasingly being investigated for their potential applications in skin health and dermatology. Previous studies demonstrated that *Streptococcus thermophilus* harbors a biologically active neutral sphingomyelinase (nSMase) capable of increasing ceramide levels in the skin. Since ceramide signaling has been implicated in the regulation of melanogenesis, the present study investigated the effects of a *Streptococcus thermophilus*-derived cell-free lysate on melanin synthesis in human epidermal melanocytes. Methods: Human epidermal melanocytes (HEMa-LP) were treated with increasing concentrations of a soluble bacterial lysate derived from *S. thermophilus* (hereafter referred to as the lysate). Cell viability, melanin content, neutral sphingomyelinase activity, and tyrosinase activity were evaluated. The involvement of nSMase signaling was investigated using the specific inhibitor GW4869. Expression of β-catenin, microphthalmia-associated transcription factor (MITF), and tyrosinase was assessed by Western blot analysis. Results: The *Streptococcus thermophilus*-derived cell-free lysate did not affect melanocyte viability but significantly reduced melanin synthesis in a concentration-dependent manner. The lysate exhibited nSMase activity, which was abolished by GW4869. Inhibition of nSMase abrogated the anti-melanogenic effect of the cell-free bacterial lysate, supporting the involvement of nSMase-dependent signaling. Treatment with the lysate induced a significant downregulation of β-catenin, MITF, and tyrosinase protein expression. In contrast, no direct inhibition of tyrosinase activity was observed in a cell-free assay. Conclusions: A *Streptococcus thermophilus*-derived cell-free lysate reduced melanogenesis in human melanocytes, an effect that is associated with nSMase activity and attenuation of the β-catenin/MITF/tyrosinase signaling pathway. These findings identify a potential strategy for the modulation of skin pigmentation and support the development of appropriate probiotic-derived approaches for the management of hyperpigmentation disorders.

Keywords: *Streptococcus thermophilus*; cell-free bacterial lysate; neutral sphingomyelinase; ceramide; melanogenesis; MITF; β-catenin; tyrosinase

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1. Introduction

Melanin synthesis is a tightly regulated physiological process that protects the skin from ultraviolet radiation and other environmental stressors. Melanocytes are highly specialized neural crest-derived cells that determine skin pigmentation through a coordinated network of signaling pathways controlling melanin synthesis, melanosome biogenesis, and melanosome transfer to neighboring keratinocytes [1,2]. Although essential for photoprotection, dysregulation of melanogenesis may lead to excessive pigment accumulation and contribute to several common dermatological disorders, including melasma, solar lentigo, and age-related hyperpigmentation [1,3,4].

Current depigmenting treatments, such as hydroquinone, retinoids, and chemical peeling agents, often show limited efficacy and may be associated with skin irritation, sensitization, or long-term safety concerns, thereby highlighting the need for innovative and biologically targeted therapeutic approaches [3–5].

In recent years, skin microbiomes have emerged as a critical regulator of cutaneous homeostasis. Human skin hosts a diverse microbial community that contributes to epidermal barrier maintenance, immune modulation, and protection against pathogenic microorganisms through dynamic host–microbe interactions [6–9].

Increasing evidence indicates that microbial-derived products can influence several aspects of skin physiology, prompting growing interest in the therapeutic exploitation of probiotics and microbiome-derived bioactive compounds [10–16]. Among these, cell-free bacterial preparations and soluble bacterial fractions obtained from beneficial microorganisms have attracted considerable attention because they retain biological activity while avoiding some of the limitations associated with the administration of live microorganisms, including issues related to viability, stability, storage, and formulation.

Recent studies have suggested that probiotic-derived products may also regulate pigmentation processes. Various bacterial strains, including species belonging to the genera *Lactobacillus*, *Lacticaseibacillus*, *Lactiplantibacillus*, *Bifidobacterium*, and *Pediococcus*, have been reported to exert anti-melanogenic activity in experimental models [17–19]. These effects have been attributed to multiple mechanisms, including inhibition of tyrosinase activity, suppression of Microphthalmia-associated Transcription Factor (MITF) expression, modulation of MAPK-dependent signaling pathways, attenuation of oxidative stress, and regulation of melanogenesis-related genes. Furthermore, several microbial metabolites and cell wall components, such as lipoteichoic acids, have been shown to reduce melanin synthesis and pigment accumulation. Collectively, these findings support the concept that probiotic-derived bioactive products can modulate pigmentation pathways and may represent promising candidates for the development of novel depigmenting agents. However, despite increasing evidence supporting the anti-melanogenic effects of probiotics and microbiome-derived bioactive products, the underlying molecular mechanisms remain only partially understood. In particular, the potential contribution of sphingolipid metabolism and ceramide signaling to the regulation of melanogenesis by probiotic-derived bacterial products has not yet been investigated.

Among bacterial species with established dermatological activity, *Streptococcus thermophilus* (*S. thermophilus*) has attracted particular attention because of its ability to increase epidermal ceramide levels through the activity of a bacterial neutral sphingomyelinase (nSMase) [15,16]. Previous studies demonstrated that *S. thermophilus* harbors a biologically active nSMase capable of hydrolyzing sphingomyelin into ceramide both in vitro and in vivo [15,16,20,21].

Moreover, topical application of preparations containing *S. thermophilus* or bacterial nSMase significantly increased ceramide levels in human skin, resulting in improved epidermal hydration and enhanced barrier function [15]. Based on these observations, we investigated

whether the nSMase activity associated with an *S. thermophilus*-derived cell-free lysate (hereafter referred to as the lysate) could contribute to the regulation of melanogenesis.

Ceramides are major structural components of the stratum corneum and play an essential role in maintaining epidermal permeability barrier integrity and skin hydration [22]. Beyond their structural functions, ceramides act as bioactive lipid mediators involved in the regulation of cell proliferation, differentiation, apoptosis, and stress responses [23]. Importantly, accumulating evidence indicates that ceramide-dependent signaling pathways also influence melanocyte biology and pigmentation. Experimental studies have shown that ceramide analogs suppress melanogenesis by reducing MITF expression and tyrosinase activity, two key determinants of melanin biosynthesis [24,25]. In addition, modulation of membrane lipid composition and sphingolipid metabolism has been reported to affect melanogenic signaling and pigment production [26]. MITF is widely recognized as the master transcriptional regulator of melanocyte differentiation and pigmentation, controlling the expression of tyrosinase and other enzymes responsible for melanin synthesis [1,27,28]. Therefore, pathways capable of modulating MITF activity represent attractive therapeutic targets for the treatment of hyperpigmentation disorders.

Given the established anti-melanogenic properties of ceramides and the ability of *S. thermophilus* to promote ceramide generation through bacterial nSMase activity, we hypothesized that an *S. thermophilus*-derived lysate may inhibit melanogenesis through nSMase-dependent signaling mechanisms. Accordingly, the aim of the present study was to investigate the effects of an *S. thermophilus*-derived cell-free lysate on melanin synthesis, tyrosinase activity, and melanogenic signaling pathways in primary human epidermal melanocytes.

By investigating the relationship between bacterial nSMase activity and melanogenic signaling, this study provides novel mechanistic insights into the potential use of probiotic-derived bioactive products for the management of hyperpigmentation disorders.

2. Materials and Methods

2.1. Preparation of the S. thermophilus Cell-Free Bacterial Lysate

Lyophilized *S. thermophilus* strain CNCM-I5570 (EOS2021, Ardea, Roma, Italy; 6×10^{11} CFU/g was washed and resuspended at 1 g/10 mL of phosphate-buffered saline (PBS). Bacterial suspensions were subjected to sonication using a Vibracell sonicator (Sonics & Materials, Danbury, CT, USA) for 30 min under alternating cycles of 10 s sonication and 10 s cooling intervals. Bacterial cell disruption was verified by measuring the absorbance of the sample at 590 nm with a spectrophotometer (Eppendorf, Hamburg, Germany) before and after every sonication step. The resulting lysates were centrifuged at 17,949× *g* for 20 min at 4 °C, and the supernatants containing soluble bacterial components were collected and filtered through sterile membrane filter, 0.22 µm pore size (Corning Incorporated, Corning, NY, USA). The filtered preparations were stored at −80 °C until use. Protein concentration was determined using the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA).

The resulting preparation was used in subsequent experiments at final protein concentrations ranging from 25 to 100 µg/mL.

2.2. Cell Culture

Primary normal human epidermal melanocytes (Human Epidermal Melanocytes, Adult–Lightly Pigmented donor; HEMa-LP, Catalog number C0245C, Thermo Fisher Scientific, Waltham, MA, USA) derived from a single adult donor were cultured in Medium 254 supplemented with PMA-Free Human Melanocyte Growth Supplement-2 (HMGS-2), gentamicin (10 µg/mL), amphotericin B (0.25 µg/mL) (Thermo Fisher Scientific), and

penicillin G (100 U/mL) (Sigma-Aldrich, St Louis, MO, USA). Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. All experiments were performed using HEMa-LP cells between passages 5 and 10.

2.3. Cell Viability Assay

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8; Sigma-Aldrich, St. Louis, MO, USA). HEMa-LP cells were seeded in 96-well plates (Corning, Corning, NY, USA) at a density of 1×10^4 cells/cm^2 and treated for 24 h with bacterial lysate (25, 50, and 100 µg/mL) or C2 ceramide (Sigma-Aldrich, St. Louis, MO, USA) dissolved in DMSO and diluted in culture medium immediately before use at concentrations of 1, 2, and 5 µM. At the end of the treatment period, the culture medium was replaced with fresh medium prior to CCK-8 reagent addition to avoid potential interference from lysate components with the assay readout. Subsequently, 10 µL of CCK-8 reagent was added to each well, and cells were incubated for two hours according to the manufacturer's protocol. Cell viability was evaluated based on optical density (OD) values measured at 450 nm using a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA).

2.4. Melanin Assay

HEMa-LP cells were seeded at a density of 1×10^5 cells/well in 6-well plates (Corning, Corning, NY, USA) and cultured until approximately 80% confluence. Cells were then treated for 24 h with C2 ceramide (1, 2, or 5 µM) or bacterial lysate (25, 50, or 100 µg/mL).

To investigate the involvement of nSMase-dependent signaling, cells were co-treated for 24 h with lysate and 5 µM GW4869 (dissolved in DMSO and diluted in culture medium immediately before use; Sigma-Aldrich, St. Louis, MO, USA), a selective inhibitor of neutral sphingomyelinase.

Cellular melanin content was quantified spectrophotometrically following alkaline extraction. Briefly, cells were harvested by trypsinization and centrifuged at 150× *g* for 10 min. Cell pellets were resuspended in 1 mL of 1 N NaOH and incubated at 80 °C for 60 min using a ThermoMixer (Eppendorf, Hamburg, Germany). Samples were then centrifuged at 16,000× *g* for 20 min at 4 °C, and the absorbance of the supernatants was measured at 405 nm using a VICTOR X4 microplate reader (PerkinElmer, Waltham, MA, USA).

Melanin concentration was determined using a standard curve generated with synthetic melanin (0–300 µg/mL; Sigma-Aldrich, St. Louis, MO, USA). To account for variations in cell number, melanin content was normalized to viable cell number determined by the Trypan Blue exclusion assay and expressed as µg melanin/10^5 cells. Cell viability assessed by Trypan Blue exclusion remained above 95% in all experimental conditions.

2.5. Sphingomyelinase Activity Assay

Neutral sphingomyelinase (nSMase) activity in the bacterial lysate was determined as previously described [20,29], with minor modifications. Briefly, protein extracts (25, 50, or 100 µg) were resuspended in a neutral reaction buffer containing 50 mM Tris-HCl (pH 7.4) and 10 mM $MgCl_2$ and pre-incubated for 30 min at 37 °C in the absence or presence of 5 µM GW4869.

The enzymatic reaction was initiated by adding 10 nmol of C12-NBD-sphingomyelin (N-[12-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]dodecanoyl]-sphingosine-1-phosphocholine; Avanti Polar Lipids, Alabaster, AL, USA) in a final reaction volume of 200 µL. Following incubation at 37 °C for 1 h, the reaction was terminated by adding 200 µL of chloroform/methanol (2:1, *v*/*v*).

Samples were centrifuged at 22,000× *g* for 10 min, and the organic phase was collected. The aqueous phase was re-extracted with 400 µL of chloroform/methanol (2:1, *v*/*v*), cen-

trifuged again, and the resulting organic phase was pooled with the previously collected lipid extract. Organic solvents were evaporated under a nitrogen stream, and lipid residues were resuspended in 80 µL of chloroform.

Samples were applied to silica gel thin-layer chromatography (TLC) plates (Merck, Kenilworth, NJ, USA) and resolved using $CHCl_3/CH_3OH/H_2O$ (65:25:4, *v*/*v*/*v*) as the mobile phase. Under these chromatographic conditions, residual NBD-sphingomyelin and the reaction product NBD-ceramide migrated with retention factor (Rf) values of 0.15 and 0.85, respectively.

Fluorescent lipid spots were visualized and quantified using a UVItec Alliance Q9 imaging system (v18.02, Cambridge, UK). Densitometric analysis was performed using the manufacturer's software. The amount of NBD-ceramide generated was calculated by interpolation on a standard calibration curve obtained using C12-NBD-ceramide (N-[12-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]dodecanoyl]-D-erythro-sphingosine; Avanti Polar Lipids, Alabaster, AL, USA). Enzyme activity was expressed as pmol ceramide generated per hour (pmol ceramide/h).

Commercial *Bacillus cereus* neutral sphingomyelinase (Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control and displayed sensitivity to GW4869 under the experimental conditions employed.

2.6. Western Blot Analysis

HEMa-LP cells treated with *S. thermophilus* lysate at the indicated concentrations were lysed in ice-cold RIPA buffer (Merck KGaA, Darmstadt, Germany) supplemented with a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA). Cell lysates were centrifuged at 17,949× *g* for 20 min at 4 °C, and protein concentrations in the supernatants were determined using the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA, USA).

Equal amounts of protein (20 µg/lane) were separated by 12.5% SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes (Bio-Rad Laboratories) according to standard procedures. Membranes were blocked with 5% non-fat dry milk in Tris-buffered saline containing 0.5% Tween-20 (TBS-T) for 1 h at room temperature and then incubated overnight at 4 °C with the following primary antibodies: rabbit anti-human β-catenin monoclonal antibody (Cell Signaling Technology, Danvers, MA, USA; 1:1000), goat anti-human MITF polyclonal antibody (Santa Cruz Biotechnology, Dallas, TX, USA), goat anti-human tyrosinase polyclonal antibody (Santa Cruz Biotechnology, Dallas, TX, USA), and mouse anti-human GAPDH monoclonal antibody (Immunological Sciences, Rome, Italy; 1:1000).

After washing, membranes were incubated for 1 h at room temperature with horseradish peroxidase (HRP)-conjugated anti-rabbit, anti-goat, or anti-mouse IgG secondary antibodies (1:2000; Immunological Sciences, Rome, Italy). Immunoreactive bands were visualized using ECL Western Blotting Chemiluminescent Substrate (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Chemiluminescence signals were acquired using an ALLIANCE imaging system (UVITEC, Cambridge, UK), and densitometric analysis was performed using the manufacturer's software. Protein expression levels were normalized to GAPDH and expressed relative to untreated control cells.

2.7. Cell-Free Tyrosinase Assay

To determine whether the bacterial lysate directly affects tyrosinase activity, 20 µL mushroom tyrosinase (1000 U/ml) was incubated with L-DOPA (2 mg/mL; 100 µL/well)

in phosphate-buffered saline (PBS, pH 7.4) in the absence or presence of lysate at concentrations of 25, 50, or 100 µg/mL.

The reaction mixture (250 µL) was incubated at 37 °C for 20 min, and dopachrome formation was quantified by measuring absorbance at 475 nm using a microplate reader. Baseline absorbance was measured immediately before incubation (T0) and subtracted from the absorbance recorded after incubation. Tyrosinase activity was expressed as a percentage of the untreated control. Mushroom tyrosinase and L-DOPA were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.8. Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 8.02 (GraphPad Software, San Diego, CA, USA). Data are presented as mean ± SEM of three independent experiments, each performed in triplicate. Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA), followed by Dunnett's or Tukey's multiple-comparison test as specified in figure legends. Differences were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Effects of Bacterial Lysate on Melanocyte Viability

The effects of lysate and C2 ceramide on HEMa-LP cell viability were evaluated after 24 h of treatment using the CCK-8 assay. As shown in Figure 1, neither the bacterial lysate (25, 50, and 100 µg/mL) nor C2 ceramide (1, 2, and 5 µM) significantly affected cell viability compared with untreated control cells.

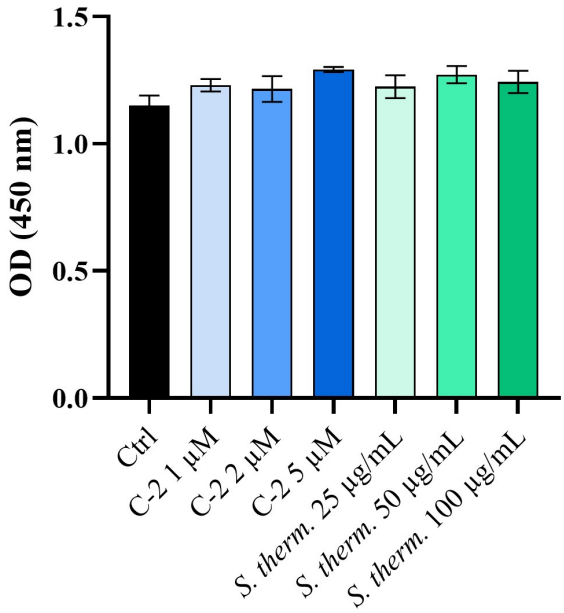

Figure 1. Effects of *S. thermophilus*-derived cell-free lysate and C2 ceramide on HEMa-LP cell viability. HEMa-LP melanocytes were treated for 24 h with lysate (25, 50, and 100 µg/mL) or C2 ceramide (1, 2, and 5 µM). Cell viability was assessed using the Cell Counting Kit-8 (CCK-8) assay and expressed as optical density (OD) values measured at 450 nm. Data are presented as mean ± SEM of three independent experiments performed in triplicate. No significant differences were observed compared with untreated control cells.

3.2. The S. thermophilus-Derived Cell-Free Lysate Inhibits Melanin Synthesis

Treatment of HEMa-LP melanocytes with lysate significantly reduced melanin content in a concentration-dependent manner (Figure 2A). A significant inhibitory effect was

already observed at the lowest concentration tested; the highest concentration evaluated (100 µg/mL) reduced melanin levels by approximately 45% compared with untreated control cells.

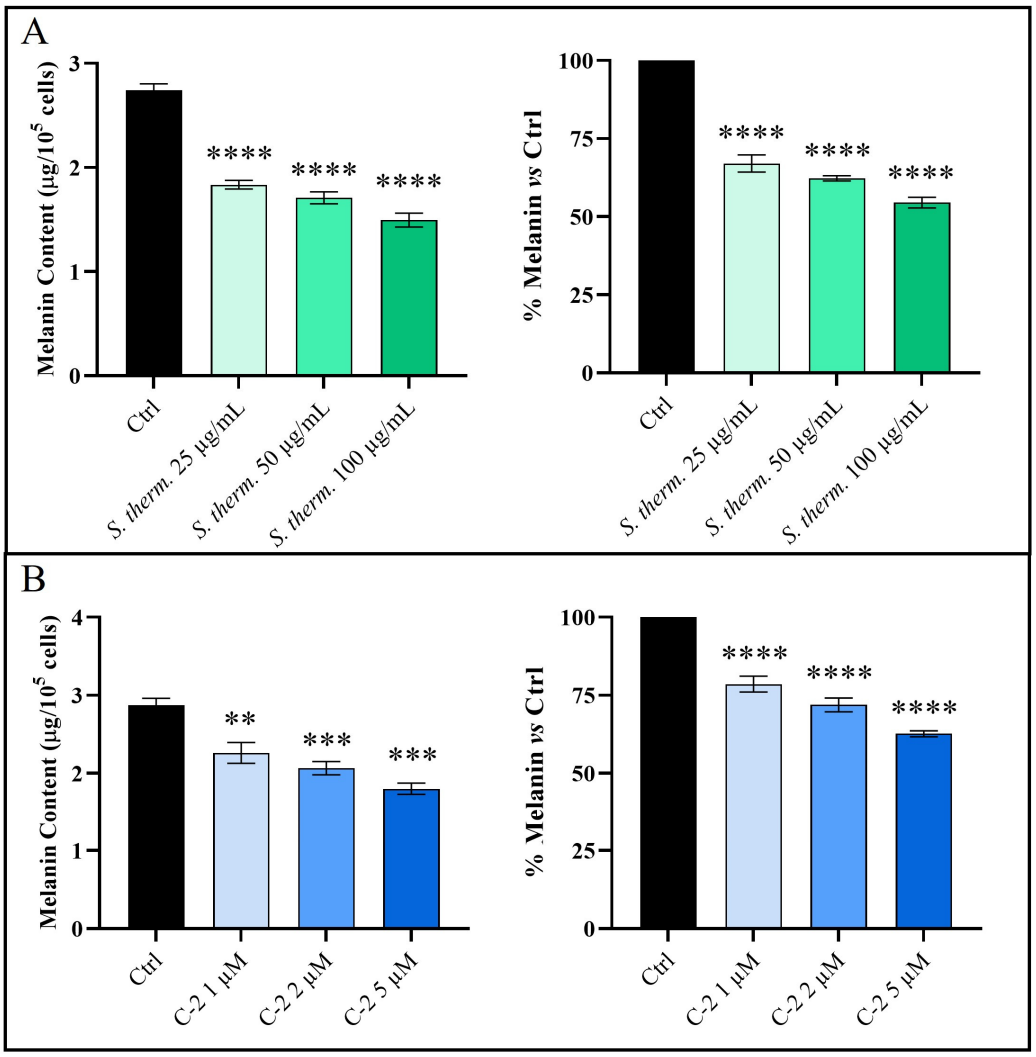

Figure 2. Effects of *S. thermophilus*-derived cell-free lysate and C2 ceramide on melanin synthesis in HEMa-LP melanocytes. (**A**) Melanin content following treatment with lysate (25, 50, and 100 µg protein/mL). The left panel shows melanin content expressed as µg melanin/10^5 cells, while the right panel shows melanin content (%) relative to untreated control cells. (**B**) Melanin content following treatment with C2 ceramide (1, 2, and 5 µM), used as a positive control. The left panel shows melanin content expressed as µg melanin/10^5 cells, while the right panel shows melanin content (%) relative to untreated control cells. Data are presented as mean ± SEM of three independent experiments, each performed in triplicate. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple-comparison test. (** $p \leq 0.01$, *** $p \leq 0.001$, and **** $p \leq 0.0001$ versus untreated control cells).

C2 ceramide was used as a positive control based on its well-established anti-melanogenic activity. Consistent with previous reports [24,25], C2 ceramide induced a concentration-dependent reduction in melanin content, confirming the responsiveness and suitability of the experimental model (Figure 2B).

3.3. Involvement of Neutral Sphingomyelinase Signaling in the Anti-Melanogenic Activity of the S. thermophilus-Derived Lysate

To determine whether the bacterial lysate preparation retained nSMase activity, NBD-sphingomyelin hydrolysis was assessed. As shown in Figure 3A, the *S. thermophilus*-derived lysate exhibited measurable nSMase activity, resulting in a concentration-dependent increase in NBD-ceramide generation. These results indicate that biologically active nSMase was retained in the lysate.

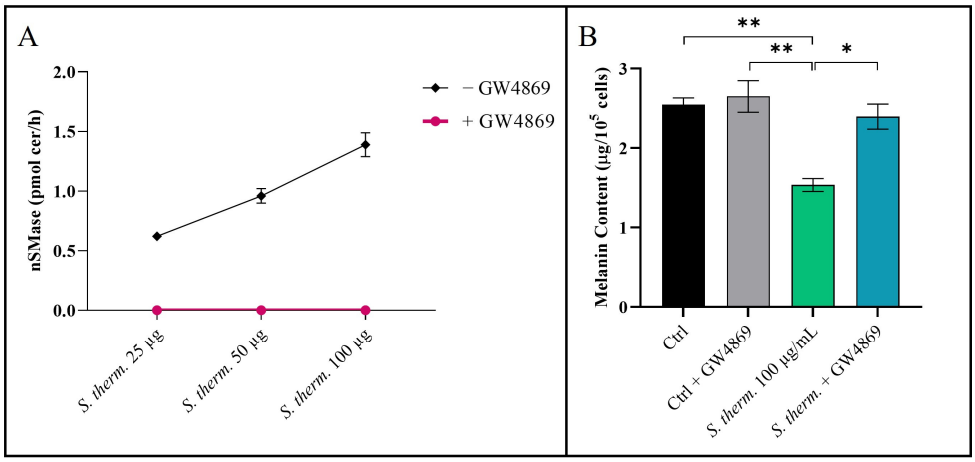

Figure 3. Evidence supporting the involvement of nSMase signaling in the anti-melanogenic activity of *S. thermophilus*-derived cell-free lysate. (**A**) Neutral SMase activity of the lysate preparation at 25, 50 and 100 µg, evaluated in the absence or presence of GW4869. Data are expressed as pmol NBD-ceramide/h and represent mean ± SEM of three independent experiments. (**B**) Effect of the nSMase inhibitor GW4869 on bacterial lysate-induced inhibition of melanogenesis. HEMa-LP melanocytes were treated with lysate (100 µg/mL) in the presence or absence of GW4869 (5 µM). Melanin content was expressed as µg melanin/10^5 cells. Data are presented as mean ± SEM of three independent experiments, each performed in triplicate. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple-comparison test (* $p \leq 0.05$; ** $p \leq 0.01$).

To investigate the involvement of nSMase signaling in the anti-melanogenic activity of the lysate, HEMa-LP melanocytes were treated with the lysate in the presence of GW4869, a selective nSMase inhibitor. As shown in Figure 3B, GW4869 significantly attenuated the reduction in melanin content induced by the lysate. The restoration of melanin levels to values comparable to untreated controls following nSMase inhibition supports the involvement of nSMase-dependent signaling in the anti-melanogenic activity of the *S. thermophilus*-derived lysate.

3.4. S. thermophilus-Derived Cell-Free Lysate Induced Downregulation of Melanogenic Signaling

To investigate the molecular mechanisms underlying the anti-melanogenic activity of the bacterial lysate preparation, the expression of β-catenin, MITF, and tyrosinase was evaluated by Western blot analysis after 24 h of treatment. As shown in Figure 4A, lysate treatment induced a concentration-dependent reduction in β-catenin expression. Consistent with this observation, the expression levels of MITF and its downstream target tyrosinase were progressively reduced following treatment with increasing concentrations of the lysate (Figure 4B,C). Collectively, these findings indicate that the lysate preparation suppresses melanogenesis-associated signaling through downregulation of the β-catenin/MITF/tyrosinase pathway.

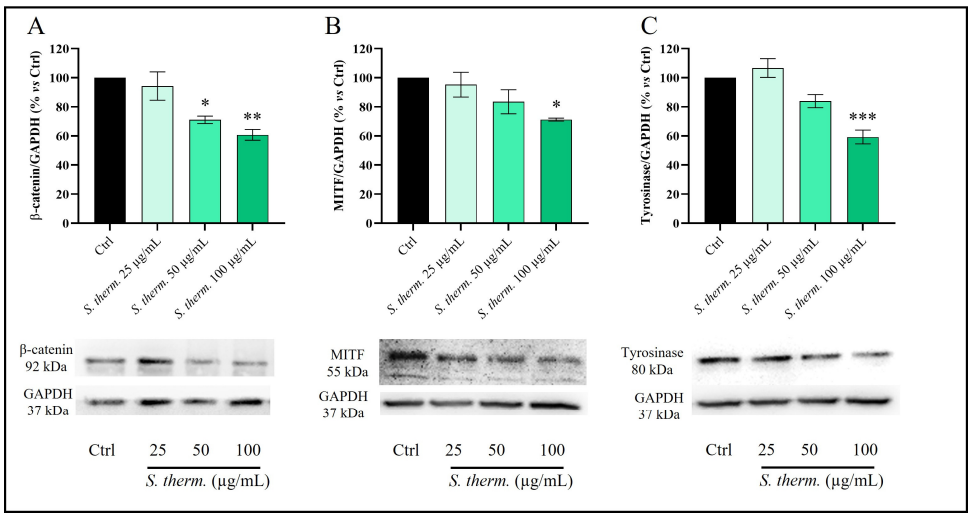

Figure 4. *S. thermophilus*-derived cell-free lysate suppresses the β-catenin/MITF/tyrosinase melanogenic pathway. Representative Western blots and densitometric analyses of β-catenin (**A**), MITF (**B**), and tyrosinase (**C**) expression in HEMa-LP melanocytes following 24 h treatment with lysate (25, 50, and 100 µg/mL). Protein expression levels were normalized to GAPDH and expressed relative to untreated control cells. Data are presented as mean ± SEM of three independent experiments. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple-comparison test. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ versus untreated control cells.

3.5. The Anti-Melanogenic Effect of S. thermophilus-Derived Cell-Free Lysate Occurs Independently of Direct Tyrosinase Inhibition

To determine whether the reduction in melanin synthesis was associated with direct inhibition of tyrosinase activity, a cell-free mushroom tyrosinase assay was performed. As shown in Figure 5, bacterial lysate did not significantly affect tyrosinase activity at any of the concentrations tested. These findings indicate that the reduction in melanin content observed in lysate-treated melanocytes is unlikely to result from direct tyrosinase inhibition and instead supports the involvement of intracellular melanogenic signaling pathways.

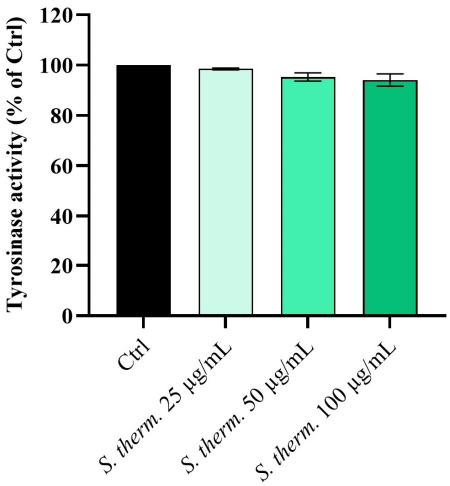

Figure 5. *S. thermophilus*-derived cell-free lysate does not affect tyrosinase activity in a cell-free system. Mushroom tyrosinase was incubated with L-DOPA in the absence or presence of lysate (25, 50, and 100 µg/mL). Tyrosinase activity was expressed as a percentage of untreated control. Data are presented as mean ± SEM of three independent experiments, each performed in triplicate. No significant differences were observed among the experimental groups.

4. Discussion

The present study demonstrates that a cell-free lysate derived from *S. thermophilus* significantly inhibits melanogenesis in human epidermal melanocytes. The reduction in cellular melanin content occurred in a concentration-dependent manner and was accompanied by downregulation of β-catenin, MITF, and tyrosinase expression, suggesting coordinated suppression of the melanogenic machinery. Furthermore, the anti-melanogenic activity was abrogated by the nSMase inhibitor GW4869, indicating the involvement of SMase-dependent signaling pathways. These findings suggest that a cell-free lysate preparation obtained from *S. thermophilus* may contribute to regulating pigmentation through modulation of intracellular signaling networks rather than through direct inhibition of tyrosinase activity.

Melanogenesis is a tightly regulated biological process controlled by multiple signaling pathways that converge on microphthalmia-associated transcription factor (MITF), the master regulator of melanocyte differentiation and pigmentation [1,2].

MITF governs the transcription of tyrosinase, tyrosinase-related protein 1 (TRP-1), and tyrosinase-related protein 2 (TRP-2), which are essential enzymes involved in melanin biosynthesis [1,27]. Consequently, modulation of MITF expression represents one of the most effective mechanisms for regulating pigment production. In the present study, treatment with the *S. thermophilus* lysate induced a marked reduction in MITF and tyrosinase protein levels, indicating that the decrease in melanin content was associated with a reduction of melanogenic gene expression. This interpretation is further supported by the cell-free assay showing the absence of direct inhibition of tyrosinase activity.

The present findings also extend the growing body of evidence supporting a role for probiotics and probiotic-derived bioactive products in the regulation of skin pigmentation. Several bacterial strains, including *Lactobacillus*, *Lacticaseibacillus*, *Lactiplantibacillus*, *Bifidobacterium*, and *Pediococcus* spp., have been reported to reduce melanogenesis through modulation of oxidative stress, suppression of MITF expression, inhibition of tyrosinase activity, and regulation of melanogenesis-related signaling pathways [17–19]. More recently, bacterial metabolites and structural components such as lipoteichoic acids have also been shown to exert anti-melanogenic effects. However, despite increasing evidence supporting the influence of probiotic-derived products on pigmentation [17], the molecular mechanisms underlying these effects remain incompletely understood. In particular, no previous studies have specifically investigated whether bacterial SMase activity and subsequent ceramide generation contribute to melanogenesis regulation. Therefore, the present study provides evidence supporting a mechanistic link between probiotic-derived bacterial products, sphingolipid metabolism, and the regulation of melanogenesis. In this context, an important finding of this study is the involvement of SMase-dependent signaling. Previous investigations from our group demonstrated that *S. thermophilus* possesses biologically active nSMase capable of hydrolyzing sphingomyelin into ceramide both in vitro and in vivo [15,16,20,21]. Topical application of preparations containing *S. thermophilus* has been associated with increased ceramide content in the stratum corneum and improved epidermal barrier function [15,16]. In the present work, the probiotic lysate preparation retained measurable SMase activity, and inhibition of this activity by GW4869 significantly attenuated its anti-melanogenic effect.

The restoration of melanin levels to values comparable to untreated controls following GW4869 treatment supports the involvement of nSMase-dependent signaling in the anti-melanogenic activity of bacterial lysate preparation.

Ceramides are well established as major structural components of the stratum corneum and play essential roles in maintaining epidermal barrier integrity and skin hydration [22,30]. Beyond these structural functions, ceramides act as bioactive lipid mediators involved in the regulation of proliferation, differentiation, apoptosis, inflammation,

and immune responses [23,31–34]. The central position of ceramide within sphingolipid metabolism supports its role as an important signaling hub capable of influencing numerous physiological pathways. On this basis, the mechanistic hypothesis explored in the present study concerns their potential function as intracellular signaling mediators involved in melanogenesis regulation.

The present findings are consistent with previous reports demonstrating that ceramides negatively regulate melanogenesis. Several studies have shown that ceramides may suppress melanogenesis through Akt-dependent pathways, promoting MITF degradation and reducing expression of downstream melanogenic enzymes [24,35]. In particular, Kim et al. reported that ceramide decreases melanin synthesis through modulation of Akt signaling [24]. Subsequently, Jeong et al. demonstrated that ceramide suppresses melanogenesis through degradation of MITF and tyrosinase [25]. More recently, ceramide administration has been shown to attenuate UVB-induced hyperpigmentation and downregulate MITF-dependent melanogenic pathways in vivo [28,36].

In our experimental model, the anti-melanogenic activity of the *S. thermophilus*-derived cell-free lysate closely resembled the response induced by exogenous C2 ceramide. Moreover, pharmacological inhibition of SMase activity substantially reversed the reduction in melanin content induced by the bacterial lysate preparation. Taken together, these observations are consistent with the hypothesis that endogenous ceramide generation downstream of bacterial SMase activity may contribute to the anti-melanogenic effect observed in the present study. Although intracellular ceramide levels were not directly quantified, several mechanisms could potentially explain this association [33,34].

One potential mechanism emerging from our results involves the modulation of β-catenin signaling. β-catenin is a key component of the canonical Wnt signaling pathway and contributes to melanocyte differentiation and pigmentation by stimulating MITF transcription. Activation of the Wnt/β-catenin pathway promotes melanogenesis, whereas suppression of β-catenin signaling is associated with reduced pigment production [2,37,38]. In our study, the bacterial lysate treatment reduced β-catenin expression. The observed decrease in β-catenin levels may provide a mechanistic link between SMase-dependent signaling and downstream inhibition of MITF and tyrosinase expression. It is therefore conceivable that nSMase activity associated with the bacterial lysate alters local sphingolipid metabolism, generating signaling molecules capable of negatively regulating the β-catenin/MITF axis. While this interpretation remains speculative, it is consistent with the inhibitory effect of GW4869 and with the similarities observed between the lysate and exogenous ceramide treatment.

Although the precise molecular interactions remain to be elucidated, the data suggest that *S. thermophilus*-derived bioactive compounds may interfere with upstream melanogenic regulators, ultimately leading to suppression of the β-catenin/MITF axis.

Interestingly, the bacterial lysate preparation did not directly inhibit tyrosinase activity in the cell-free assay. This observation is consistent with a previous study showing that sphingolipid-related molecules may regulate melanogenesis through transcriptional and signaling mechanisms rather than through direct enzymatic inhibition [39]. Although the assay was performed using mushroom tyrosinase rather than the human enzyme, which may have influenced the observed response, the results overall support an indirect mechanism of melanogenesis regulation. In contrast, many conventional depigmenting agents, including hydroquinone, kojic acid, and arbutin, exert their effects at least partially by directly inhibiting tyrosinase activity [3,4]. However, these compounds may be associated with skin irritation, instability, or safety concerns following prolonged use. The absence of direct tyrosinase inhibition observed in the present study suggests a distinct mode of action

based on modulation of intracellular signaling pathways. Such an upstream mechanism may offer advantages in terms of selectivity and potential reduction in toxicological concerns.

From a broader perspective, this work contributes to the growing interest in probiotic-derived bioactive products for dermatological applications. Cell-free bacterial preparations and soluble bacterial fractions derived from beneficial microorganisms have attracted considerable attention because they retain biological activity while avoiding some of the limitations associated with the use of live micro-organisms, including issues related to viability, storage, preservation, stability, and formulation. Their applications in dermatology are rapidly expanding, particularly in the fields of skin barrier repair, inflammation control, and cosmetic science. The present findings suggest that regulation of pigmentation may represent an additional and previously underexplored therapeutic target for microbiome-derived bioactive products.

Limitations and Future Perspectives. Several limitations of the present study should be acknowledged. First, the experiments were performed in cultured human melanocytes directly exposed to the *S. thermophilus*-derived cell-free lysate. While this model is suitable for investigating cellular and molecular mechanisms regulating melanogenesis, it does not reproduce the complexity of topical application to intact human skin. Factors such as epidermal barrier penetration, formulation-dependent delivery, stability of active components, and interactions with other skin cell populations were not evaluated. Second, although the lysate exhibited measurable nSMase activity and GW4869 significantly attenuated its biological effects, intracellular ceramide levels were not directly quantified. Therefore, the involvement of ceramide generation requires further experimental confirmation. Third, the experiments were designed to investigate the acute anti-melanogenic activity of the lysate and its underlying mechanisms. The effects of repeated or prolonged exposure were not evaluated and will require further investigation in more physiologically relevant experimental models. Fourth, the absence of significant effects on cell viability after 24 h indicates that the tested concentrations were not acutely cytotoxic under the experimental conditions employed. However, these findings should not be interpreted as evidence of cutaneous tolerability or long-term safety, which will require evaluation in reconstructed human skin models, ex vivo tissues, and clinical studies. Finally, additional melanogenic markers, including TRP-1, TRP-2 [40], were not investigated and may provide further mechanistic insight. Despite these limitations, the present study identifies a previously unrecognized role for *S. thermophilus*-derived cell-free lysate in the regulation of human pigmentation. To our knowledge, this is among the first studies to suggest the involvement of bacterial nSMase activity and ceramide-associated signaling in the anti-melanogenic effects of probiotic-derived cell-free bacterial preparation. These findings provide further support for the role of sphingolipid metabolism in the regulation of melanogenesis and identify a mechanistic pathway that may be relevant to pigmentation control in vivo. Considering the increasing demand for safe and effective therapies for hyperpigmentation disorders, the identification of an nSMase-associated anti-melanogenic pathway may provide a rationale for future investigations aimed at determining whether this mechanism contributes to the modulation of skin pigmentation in vivo and can eventually be exploited for the development of novel therapeutic approaches. Future studies should evaluate topical formulations containing the *S. thermophilus*-derived lysate in comparison with matched vehicle controls to determine whether the anti-melanogenic effects observed in cultured melanocytes translate into measurable changes in skin pigmentation under in vivo conditions. Such studies should also address formulation-dependent delivery, cutaneous tolerability, and the influence of repeated application.

5. Conclusions

The present study demonstrates that an *S. thermophilus*-derived cell-free lysate effectively reduced melanogenesis in human epidermal melanocytes. The anti-melanogenic activity was associated with SMase-dependent signaling and accompanied by downregulation of the β-catenin/MITF/tyrosinase pathway, supporting modulation of melanogenic gene expression rather than direct inhibition of tyrosinase enzymatic activity.

These findings provide novel evidence linking a probiotic-derived cell-free lysate to the regulation of pigmentation and support the hypothesis that bacterial nSMase activity may contribute to melanogenesis control through a putative ceramide-dependent mechanism. By identifying a potential role for bacterial nSMase activity and sphingolipid signaling in melanogenesis regulation, this study provides mechanistic insights that may be relevant to the development of future strategies targeting skin pigmentation. (Figure 6).

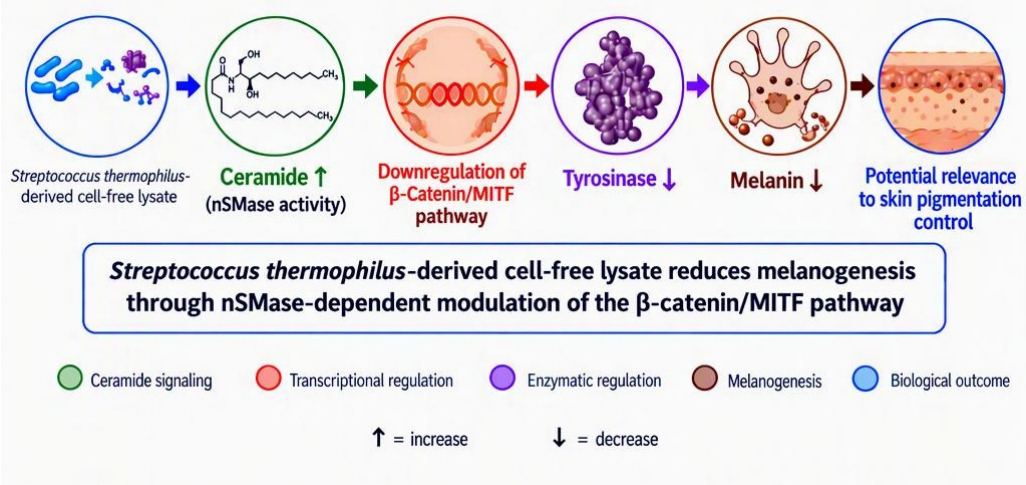

Figure 6. Proposed mechanism of action of the *Streptococcus thermophilus*-derived cell-free lysate in human melanocytes. Experimental findings support the involvement of nSMase-dependent signaling associated with attenuation of the β-catenin/MITF/tyrosinase pathway and reduced melanin synthesis.

From a translational perspective, the anti-melanogenic activity observed for the *S. thermophilus*-derived cell-free lysate highlights its potential as a source of bioactive compounds for dermatological and dermo-cosmetic applications. Considering the increasing demand for safe and effective approaches to hyperpigmentation disorders, appropriate probiotic-derived bioactive products may represent a promising new class of depigmenting agents.

Further studies using reconstructed human skin models, ex vivo tissues, and clinical investigations will be required to confirm efficacy, identify the specific bioactive components involved, directly assess ceramide generation, and establish the translational potential of these findings for the management of melasma, solar lentigines, post-inflammatory hyperpigmentation, and other pigmentary disorders.

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Abbreviations

The following abbreviations are used in this manuscript:

GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
ECL	Enhanced Chemiluminescence
HEMa-LP	Human Epidermal Melanocytes adult-Lightly Pigmented donor
IgG	Immunoglobulin G
ISAPP	International Scientific Association for Probiotics and Prebiotics
L-DOPA	L-3,4-dihydroxyphenylalanine
MAPK	Mitogen-activated protein kinase
MITF	Microphthalmia-associated Transcription Factor
SEM	Standard error mean
S. thermophilus	*Streptococcus thermophilus*
TRP	tyrosinase-related protein

References

1. Costin, G.E.; Hearing, V.J. Human skin pigmentation: Melanocytes modulate skin color in response to stress. *FASEB J.* **2007**, *21*, 976–994. [CrossRef] [PubMed]
2. Cui, Y.Z.; Man, X.Y. Biology of melanocytes in mammals. *Front. Cell Dev. Biol.* **2023**, *11*, 1309557. [CrossRef] [PubMed]
3. Briganti, S.; Camera, E.; Picardo, M. Chemical and instrumental approaches to treat hyperpigmentation. *Pigment Cell Res.* **2003**, *16*, 101–110. [CrossRef] [PubMed]
4. Solano, F.; Briganti, S.; Picardo, M.; Ghanem, G. Hypopigmenting agents: An updated review on biological, chemical and clinical aspects. *Pigment Cell Res.* **2006**, *19*, 550–571. [CrossRef] [PubMed]
5. Gan, C.; Rodrigues, M. An Update on New and Existing Treatments for the Management of Melasma. *Am. J. Clin. Dermatol.* **2024**, *25*, 717–733. [CrossRef] [PubMed]
6. Chiller, K.; Selkin, B.A.; Murakawa, G.J. Skin microflora and bacterial infections of the skin. *J. Investig. Dermatol. Symp. Proc.* **2001**, *6*, 170–174. [CrossRef] [PubMed]
7. Fredricks, D.N. Microbial ecology of human skin in health and disease. *J. Investig. Dermatol. Symp. Proc.* **2001**, *6*, 167–169. [CrossRef] [PubMed]
8. Grice, E.A.; Kong, H.H.; Renaud, G.; Young, A.C.; Program, N.C.S.; Bouffard, G.G.; Blakesley, R.W.; Wolfsberg, T.G.; Turner, M.L.; Segre, J.A. A diversity profile of the human skin microbiota. *Genome Res.* **2008**, *18*, 1043–1050. [CrossRef] [PubMed]
9. Kong, H.H. Skin microbiome: Genomics-based insights into the diversity and role of skin microbes. *Trends Mol. Med.* **2011**, *17*, 320–328. [CrossRef] [PubMed]
10. De Almeida, C.V.; Antiga, E.; Lulli, M. Oral and Topical Probiotics and Postbiotics in Skincare and Dermatological Therapy: A Concise Review. *Microorganisms* **2023**, *11*, 1420. [CrossRef] [PubMed]
11. Prajapati, S.K.; Lekkala, L.; Yadav, D.; Jain, S.; Yadav, H. Microbiome and Postbiotics in Skin Health. *Biomedicines* **2025**, *13*, 791. [CrossRef] [PubMed]
12. Augello, F.R.; Lombardi, F.; Ciafarone, A.; Altamura, S.; Marazzato, M.; Artone, S.; Cinque, B.; Palumbo, P. Streptococcus thermophilus CNCM I-5570 lysate counteracts the aging process in human dermal fibroblast cells by neutralizing harmful free radicals and impacting antioxidant and anti-inflammatory pathways, thus restoring their physiological functions. *Biomed. Pharmacother.* **2025**, *185*, 117975. [CrossRef] [PubMed]

13. Altamura, S.; Dybeli, E.; Lombardi, F.; Palumbo, P.; Augello, F.R.; Topi, S.; Cifone, M.G.; Cinque, B. The anti-inflammatory and analgesic potential of the probiotic Streptococcus thermophilus via inhibition of endocannabinoid-degrading enzymes: A preliminary in vitro study. *Explor. Immunol.* **2025**, *5*, 1003233. [CrossRef]
14. Lombardi, F.; Augello, F.R.; Artone, S.; Bahiti, B.; Sheldon, J.M.; Giuliani, M.; Cifone, M.G.; Palumbo, P.; Cinque, B. Efficacy of probiotic Streptococcus thermophilus in counteracting TGF-beta1-induced fibrotic response in normal human dermal fibroblasts. *J. Inflamm.* **2022**, *19*, 27. [CrossRef] [PubMed]
15. Di Marzio, L.; Cinque, B.; De Simone, C.; Cifone, M.G. Effect of the lactic acid bacterium Streptococcus thermophilus on ceramide levels in human keratinocytes in vitro and stratum corneum in vivo. *J. Investig. Dermatol.* **1999**, *113*, 98–106. [CrossRef] [PubMed]
16. Di Marzio, L.; Cinque, B.; Cupelli, F.; De Simone, C.; Cifone, M.G.; Giuliani, M. Increase of skin-ceramide levels in aged subjects following a short-term topical application of bacterial sphingomyelinase from Streptococcus thermophilus. *Int. J. Immunopathol. Pharmacol.* **2008**, *21*, 137–143. [CrossRef] [PubMed]
17. Katona, G.; Jovanovic Ljeskovic, N.; Strahinic, I.; Stanisavljevic, N.; Vojvodic, S.; Djuris, J.; Pavic, A. Targeting Melanogenesis with Postbiotics: An Integrated Zebrafish-Based Assessment of Lactobacillus salivarius BGHO-1 and Lactobacillus paracasei BGSJ2-8. *Molecules* **2025**, *30*, 4134. [CrossRef] [PubMed]
18. Seo, J.; Sung, E.J.; Son, D.Y.; Yoo, D.S.; Seo, D.B.; Kim, M.; Yoo, C. Anti-Photoaging and Anti-Melanogenic Effects of a Novel Lactiplantibacillus plantarum RKBP Isolated from Giant Centella asiatica. *J. Microbiol. Biotechnol.* **2026**, *36*, e2603011. [CrossRef] [PubMed]
19. Kim, S.; Seo, H.; Mahmud, H.A.; Islam, M.I.; Sultana, O.F.; Lee, Y.; Kim, M.; Song, H.Y. Melanin Bleaching and Melanogenesis Inhibition Effects of Pediococcus acidilactici PMC48 Isolated from Korean Perilla Leaf Kimchi. *J. Microbiol. Biotechnol.* **2020**, *30*, 1051–1059. [CrossRef] [PubMed]
20. Gnocchi, D.; Lombardi, F.; Cinque, B.; Paparella, R.R.; Palumbo, P.; Mazzocca, A. A lysate of a multi-strain probiotic formulation inhibits cell growth and oxidative phosphorylation in colorectal cancer cells via a ceramide-mediated mechanism. *Benef. Microbes* **2026**. *Epub ahead of printing*. [CrossRef] [PubMed]
21. Angulo, S.; Morales, A.; Danese, S.; Llacuna, L.; Masamunt, M.C.; Pultz, N.; Cifone, M.G.; De Simone, C.; Delgado, S.; Vila, J.; et al. Probiotic sonicates selectively induce mucosal immune cells apoptosis through ceramide generation via neutral sphingomyelinase. *PLoS ONE* **2011**, *6*, e16953. [CrossRef] [PubMed]
22. Madison, K.C. Barrier function of the skin: "la raison d'etre" of the epidermis. *J. Investig. Dermatol.* **2003**, *121*, 231–241. [CrossRef] [PubMed]
23. Di Nardo, A.; Benassi, L.; Magnoni, C.; Cossarizza, A.; Seidenari, S.; Giannetti, A. Ceramide 2 (N-acetyl sphingosine) is associated with reduction in Bcl-2 protein levels by Western blotting and with apoptosis in cultured human keratinocytes. *Br. J. Dermatol.* **2000**, *143*, 491–497. [CrossRef] [PubMed]
24. Kim, D.S.; Kim, S.Y.; Moon, S.J.; Chung, J.H.; Kim, K.H.; Cho, K.H.; Park, K.C. Ceramide inhibits cell proliferation through Akt/PKB inactivation and decreases melanin synthesis in Mel-Ab cells. *Pigment Cell Res.* **2001**, *14*, 110–115. [CrossRef] [PubMed]
25. Jeong, H.S.; Choi, H.R.; Yun, H.Y.; Baek, K.J.; Kwon, N.S.; Park, K.C.; Kim, D.S. Ceramide PC102 inhibits melanin synthesis via proteasomal degradation of microphthalmia-associated transcription factor and tyrosinase. *Mol. Cell. Biochem.* **2013**, *375*, 81–87. [CrossRef] [PubMed]
26. Schallreuter, K.U.; Hasse, S.; Rokos, H.; Chavan, B.; Shalbaf, M.; Spencer, J.D.; Wood, J.M. Cholesterol regulates melanogenesis in human epidermal melanocytes and melanoma cells. *Exp. Dermatol.* **2009**, *18*, 680–688. [CrossRef] [PubMed]
27. Ando, H.; Kondoh, H.; Ichihashi, M.; Hearing, V.J. Approaches to identify inhibitors of melanin biosynthesis via the quality control of tyrosinase. *J. Investig. Dermatol.* **2007**, *127*, 751–761. [CrossRef] [PubMed]
28. Wang, F.; Ma, W.; Fan, D.; Hu, J.; An, X.; Wang, Z. The biochemistry of melanogenesis: An insight into the function and mechanism of melanogenesis-related proteins. *Front. Mol. Biosci.* **2024**, *11*, 1440187. [CrossRef] [PubMed]
29. Loidl, A.; Claus, R.; Deigner, H.P.; Hermetter, A. High-precision fluorescence assay for sphingomyelinase activity of isolated enzymes and cell lysates. *J. Lipid Res.* **2002**, *43*, 815–823. [CrossRef]
30. Li, Q.; Fang, H.; Dang, E.; Wang, G. The role of ceramides in skin homeostasis and inflammatory skin diseases. *J. Dermatol. Sci.* **2020**, *97*, 2–8. [CrossRef] [PubMed]
31. Jamjoum, R.; Majumder, S.; Issleny, B.; Stiban, J. Mysterious sphingolipids: Metabolic interrelationships at the center of pathophysiology. *Front. Physiol.* **2023**, *14*, 1229108. [CrossRef] [PubMed]
32. Lee, M.; Lee, S.Y.; Bae, Y.S. Functional roles of sphingolipids in immunity and their implication in disease. *Exp. Mol. Med.* **2023**, *55*, 1110–1130. [CrossRef] [PubMed]
33. Stith, J.L.; Velazquez, F.N.; Obeid, L.M. Advances in determining signaling mechanisms of ceramide and role in disease. *J. Lipid Res.* **2019**, *60*, 913–918. [CrossRef] [PubMed]
34. Uchida, Y. Ceramide signaling in mammalian epidermis. *Biochim. Biophys. Acta* **2014**, *1841*, 453–462. [CrossRef] [PubMed]
35. Kim, D.S.; Kim, S.Y.; Chung, J.H.; Kim, K.H.; Eun, H.C.; Park, K.C. Delayed ERK activation by ceramide reduces melanin synthesis in human melanocytes. *Cell Signal* **2002**, *14*, 779–785. [CrossRef] [PubMed]

36. Zang, L.; Kagotani, K.; Hayakawa, T.; Kagawa, T.; Fujimoto, Y.; Hayashi, A.; Shimada, Y.; Nishimura, N. Oral ceramide attenuates ultraviolet Binduced epidermal dysregulation and hyperpigmentation by modulating MITF signaling and barrier-related pathways. *Biomed. Pharmacother.* **2026**, *201*, 119590. [CrossRef] [PubMed]
37. Duan, X.; Hu, Y.; Fu, C.; Chen, J.; Huang, J.; Dai, X.; Zhao, Y.; Jiang, L.; Zeng, Q. Peroxisome Membrane Protein PEX16 Inhibits Melanogenesis by Inhibiting the Wnt/beta-Catenin Signalling Pathway. *Exp. Dermatol.* **2026**, *35*, e70204. [CrossRef] [PubMed]
38. Zhang, Y.; Zhang, F.; Yu, X.; Zhao, X.; Chen, J.; Zhu, J.; Kang, L. CXCL1-CXCR2 axis inhibits melanogenesis through the suppression of the WNT/beta-catenin pathway. *Front. Immunol.* **2026**, *17*, 1907373. [CrossRef] [PubMed]
39. Tokudome, Y.; Fukutomi, M. Sphingomyelin reduces melanogenesis in murine B16 melanoma cells through indirect suppression of tyrosinase. *Cytotechnology* **2023**, *75*, 93–101. [CrossRef] [PubMed]
40. Li, C.; Kuai, L.; Cui, R.; Miao, X. Melanogenesis and the Targeted Therapy of Melanoma. *Biomolecules* **2022**, *12*, 1874. [CrossRef] [PubMed]

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