# Research on UV Adaptive Skin Flora Metabolites on Human Keratinocytes

**Abstract:**

**Objectives**:

Identify UV-adapted skin surface bacteria and investigate whether their metabolites can protect UV-damaged human keratinocytes (HaCaT) and explore the underlying mechanisms.

**Methods**:

Using the SRA database, we conducted a statistical analysis of UV-stress-related skin microbiota to identify UV-adapted strains. These strains were cultured, and their metabolites were obtained via ultrasonic lysis. HaCaT cells were cultured at 37°C in a 5% CO2 incubator until they reached the logarithmic growth phase. Then, cells were divided into groups of control (untreated), UVB exposure (UVB irradiation at 200 mJ/cm²), and UVB exposure with metabolite treatment (200 mJ/cm² + 20 mg/mL). After treatment, cell viability was assessed by MTT assay, and ROS and SOD levels were measured. Besides, total protein was extracted from each group, and Western blot was applied to analyze the expression of NF-κB signaling pathway-related proteins.

**Results**:

1. Analysis of the relative abundance of skin surface bacteria before and after UV-exposure identified *Sphingomonas mucosissima* as a UV-adapted strain.
2. UVB treatment significantly reduced HaCaT cell viability and increased ROS and SOD levels, that proved the photodamage model to be reliable.
3. Treatment with *Sphingomonas mucosissima* metabolites increased cell viability and decreased ROS and SOD levels compared to the UVB-exposed group. The UVB-treated group showed upregulation of NF-κB signaling pathway expression, while *Sphingomonas mucosissima* metabolites inhibited UVB-activated NF-κB expression, which is likely due to *Sphingomonas mucosissima* metabolites.

**Keywords**: *Sphingomonas mucosissima*; Metabolites; Human Keratinocytes; Photodamage

## 1. Introduction

With the development of medical cosmetic technology, the issue of skin aging caused by exogenous factors, such as ultraviolet (UV) radiation, has gained widespread attention from both the public and professionals. Among these factors, the impact of UV radiation on skin health has been a major focus of research.

Serving as the body's first line of defense against the external environment, the skin is continuously exposed to UV radiation. UV radiation is categorized into three bands based on wavelength: UVA (320-400 nm), UVB (280-320 nm), and UVC (200-290 nm). Among these, UVA and UVB can penetrate through the Earth's atmosphere and reach the surface. UVB is primarily absorbed by the epidermis, due to its shorter wavelength and higher photon energy. Whereas UVA, with greater penetration ability, can reach the skin dermis. UVB affects keratinocytes in the epidermis, leading to direct or indirect damage such as DNA damage and mutations, impaired protein synthesis, accumulation of reactive oxygen species (ROS), altered metabolic enzyme activity, collagenase activation, and abnormal secretion of cytokines and growth factors. These damage responses can ultimately result in sunburn, immunosuppression, photoaging, and even skin cancer.[1]

Over the years, people extract photodamage-protective components from plants. Compounds such

as resveratrol, tea polyphenols, and salidroside have been verified to have protective effects against photodamage and have been successfully incorporated into skincare products. Various alkaloids, polyphenols, saponins, and polysaccharides have been shown to prevent and protect against photoaging through different mechanisms, such as scavenging free radicals, inhibiting apoptosis, reducing skin inflammation, and enhancing skin resistance. However, the application of these natural extracts faces challenges, including significant toxicity and complex extraction processes, impeding their practical use.[2]

In recent years, research on skin microbiota has expanded our knowledge of skin health. There is a complex interaction between skin microbiota and skin health, which plays a crucial role in maintaining skin health and defending against skin diseases, such as protecting the skin from pathogens, regulating immune responses, promoting wound healing, and maintaining skin barrier function. Studies have shown that skin commensal bacteria can protect the skin from pathogens by secreting antimicrobial peptides (AMPs). For instance, 6-HAP produced by Staphylococcus epidermidis strains can reduce the incidence of UV-induced tumors. These findings suggest that skin microbiota not only help maintain homeostasis by stabilizing microbial populations but also influence skin health through their metabolites.[3]

Research integrating with 16S rRNA sequencing has explored differences in skin microbiota before and after sun exposure. After four weeks of sun exposure, there were significant changes in the β-diversity of the microbiota on the forearms of participants, indicating the impact of sunlight exposure on microbial diversity and composition. Additionally, low-abundance species showed more pronounced differences before and after sun exposure. We hypothesize that species with increased abundance after sun exposure may have certain UV-adaptive characteristics and play a regulatory role in skin health.[4][5]

This study uses the SRA database and applied linear discriminant analysis (LDA) and effect size estimation to identify UV-adapted skin surface microorganisms. Their metabolites are then tested for protective functions in a human keratinocyte UVB damage model.

## 2. Experimental Methods

### 2.1 Data Analysis

The dataset was from the accession number PRJNA 987584 in the SRA database. For amplicon sequence variants (ASVs), sequence alignment was performed using the q2-alignment plugin, combined with the q2-classifier and 97% similarity ASV reference sequences from the Greengenes database (version 13.8) to construct a BIOM format table with sample information. ASVs detected in negative control samples were excluded from the results. The data were then imported into the qiime2 R package and subsequently analyzed using the Phyloseq package, eventually converting the data into relative abundance tables for each sample.

### 2.2 Bacterial Culture

The *Sphingomonas mucosissima* strain, stored in glycerol tubes at -80°C (purchased from TestBio Biological Technology), was revived and activated by two consecutive inoculations on LB solid medium. A single colony was transferred to 100 mL of LB liquid medium and cultured at 30°C with shaking at 120 rpm for 16 hours. The bacterial postponement was then collected and centrifuged. The precipitate was washed with PBS buffer and exposed to ultrasonic lysis at 1200 Hz for 10 minutes. After centrifuging at 5000 rpm for 5 minutes, the supernatant was collected as the *Sphingomonas mucosissima* metabolite sample.

### 2.3 Cell Culture and Photodamage Model Establishment

HaCaT cells were cultured in a 37°C incubator with 5% CO2. When cell confluence exceeded 90%, we carried out subculturing. The cells were treated with 0.5% trypsin and seeded in 60 mm and 90 mm culture dishes or 96-well plates at appropriate densities using high-glucose DMEM medium. HaCaT cells were seeded in 60 mm culture dishes, and when 80% confluent, a 20W UVB lamp was suspended above the cells in a clean bench for irradiation at a dose of 200 mJ/cm².

### 2.4 Apoptosis Detection

Cell viability was assessed using the MTT assay. After UV irradiation and metabolite treatment for 24 hours in 96-well plates, 20 μL of 5 mg/mL MTT solution was added to each well. The plates were stored at 37°C in the dark for 4 hours. The supernatant was then removed, and 200 μL of DMSO were added to each well, followed by shaking at room temperature for 10 minutes to dissolve the formazan crystals. Absorbance was measured at 492 nm using a microplate reader, and the relative absorbance values were collected to evaluate apoptosis.

### 2.5 Protein Expression Detection

Protein expression levels were analyzed using Western blot (WB). Cells were lysed with RIPA buffer, and the supernatant containing total protein was collected after centrifugation at 150,000 g for 15 minutes. Protein concentration was determined using the BCA method. A 30 μg sample was subjected to gel electrophoresis and transferred to a membrane. The membrane was preserved overnight at 4°C with the primary antibody (β-Tubulin), followed by incubation with the secondary antibody at room temperature for 1 hour the next day. Finally, gel imaging and recording were performed.

## 3. Results

### 3.1 Increased Relative Abundance of *Sphingomonas mucosissima* After UV Exposure

The study by Willmott et al. investigated the impact of UV exposure on the skin microbiota, collecting data from 21 healthy volunteers before and after sun exposure in a sun-rich environment. This dataset, available on the SRA database under accession number PRJNA 987584, was analyzed to explore the differences in skin microbiota following UV exposure. Using the LEfSe method, we identified significant increases in certain bacterial species, including a more than 3.5-fold increase in *Sphingomonas mucosissima* post-exposure (Figure 1). This suggests that these bacteria may have a strong UV adaptation capability, that can enhance the skin's resilience to UV radiation. Literature review indicates that Sphingomonas species can produce ubiquinone-10, sphingolipids, and carotenoids, which is known for their antioxidant properties. Given that UV exposure raises reactive oxygen species (ROS) levels in skin cells, leading to mutations and inflammatory responses, we hypothesize that *Sphingomonas mucosissima* metabolites might offer protective effects against UV-induced skin damage.[4]

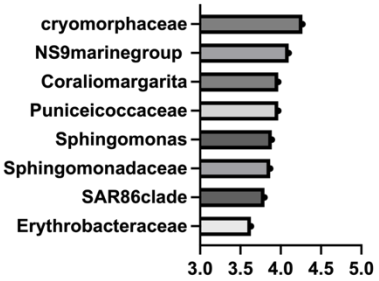

Figure 1: Effect of UV Exposure on the Abundance of Specific Bacterial Species in Skin Microbiota

### 3.2 *Sphingomonas mucosissima* Metabolites Reduce UV-Induced Apoptosis and Oxidative Damage in HaCaT Cells

Cells were divided into three groups: control, UVB exposure, and UVB exposure plus *Sphingomonas mucosissima* metabolite treatment. Each group included a 96-well plate for viability and oxidative stress marker assays and a 6-well plate for protein extraction and subsequent detection. HaCaT cells were seeded and, upon reaching 80% confluence, treated with 20 mg/mL *Sphingomonas mucosissima* metabolites for the treatment group. The control plates were wrapped in aluminum foil, while the rest were radiated under a 20W UVB lamp at 200 mJ/cm² for 20 minutes. Following 24 hours of incubation, we observed a significant reduction in cell numbers post-UVB exposure, which was mitigated by the *Sphingomonas mucosissima* metabolite treatment (Figure 2). MTT assay results indicated that cell viability significantly decreased after UVB exposure but was notably higher in the treatment group compared to the UVB-only group (Figure 3). This indicates that the metabolites reduce UV-induced apoptosis in HaCaT cells.

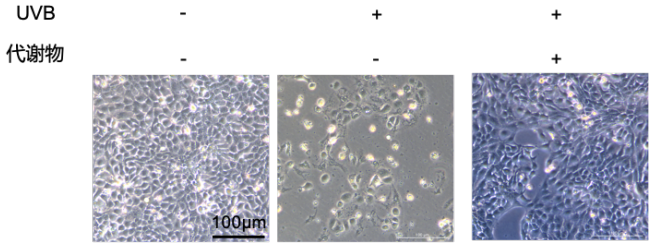


Figure 2: Effect of *Sphingomonas mucosissima* Metabolites on Cell Morphology Post-UVB Irradiation

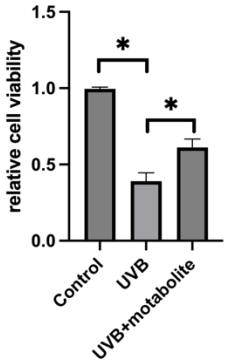


Figure 3: Effect of *Sphingomonas mucosissima* Metabolites on Cell Viability Post-UVB Irradiation

ROS levels, a key indicator of oxidative stress, significantly increased on post-UVB exposure but decreased markedly with metabolite treatment, showcasing their antioxidant capacity (Figure 4).

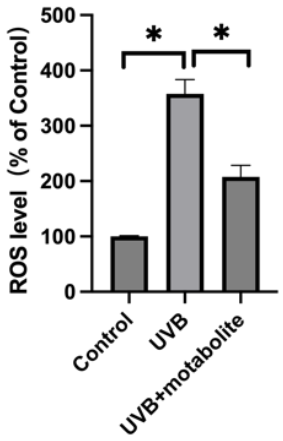


Figure 4: Effect of *Sphingomonas mucosissima* Metabolites on ROS Levels Post-UVB Irradiation

Superoxide dismutase (SOD), a crucial antioxidant enzyme, notably reduced in the UVB exposure group, representing heightened oxidative stress. Metabolite treatment restored SOD levels, demonstrating their protective antioxidant effects (Figure 5).

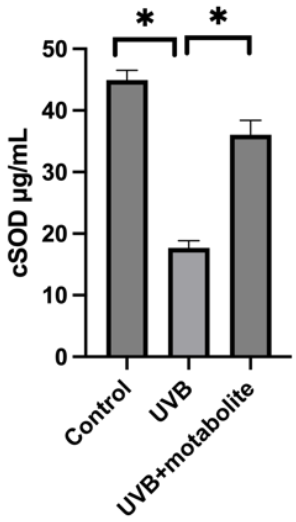


Figure 5: Effect of *Sphingomonas mucosissima* Metabolites on SOD Levels Post-UVB Irradiation

**3.3 *Sphingomonas mucosissima* Metabolites Inhibit UVB-Activated NF-κB Signaling Pathway**

To explain the protective mechanisms of *Sphingomonas mucosissima* metabolites, we measured key proteins in common damage pathways. UVB exposure markedly upregulated IκBα and p65, indicating activation of the NF-κB pathway, which is linked to apoptosis and inflammation. Metabolite treatment reduced IκBα and p65 expression levels, suggesting that these metabolites can inhibit the NF-κB signaling pathway, thereby mitigating UV-induced damage (Figure 6).

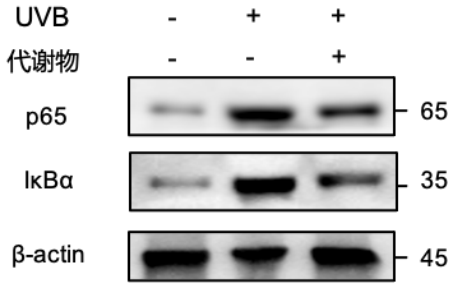


Figure 6: Inhibition of UVB-Induced NF-κB Pathway Activation by *Sphingomonas mucosissima*

**4. Discussion**

UVB radiation significantly impacts human health, with prolonged exposure disrupting the redox state of skin cells, inducing inflammation, inhibiting cell proliferation, and potentially leading to apoptosis. While most research focuses on natural substances that offer protection or repair from UVB-induced photodamage, the function of skin microbiota in this context is rarely reported. The skin, acting as the interface between the body and external environment, serves as a physical, chemical, and immunological barrier. It also hosts a microbiota that collectively forms the body's first line of defense against external threats and pathogens. Over time, the skin microbiota co-evolves with the host, contributing to maintaining health by defending against harmful factors. Therefore, the skin microbiota is closely associated with skin health issues.[3]

Nakatsuji et al. discovered a specific S. epidermidis strain that produces 6-N-hydroxyaminopurine (6-HAP), which protects epidermal cells from keratinocyte carcinoma in a photodamage mouse model. Administering 6-HAP intravenously in mice effectively inhibited the growth of B16 F10 melanoma cells without significant systemic toxicity. 6-HAP inhibits DNA polymerase activity and tumor cell proliferation without affecting normal keratinocyte proliferation, offering a potential strategy that skin bacteria can moderate skin health through metabolite secretion.[7]

Inspired by this, we identified *Sphingomonas mucosissima*, a skin bacterium thriving under intense UV exposure. Cultivating this bacterium in vitro and enriching its metabolites, we used these metabolites to treat UVB-damaged HaCaT cells. We found that the metabolites reduced oxidative stress and regulated the NF-κB signaling pathway associated with apoptosis and inflammation in HaCaT cells, offering broad protection post-UVB exposure.

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The NF-κB pathway is a complex signaling network. In its inactive state, NF-κB is isolated in the cytoplasm by IκB proteins. ROS accumulation activates NF-κB, prompting the p65 subunit to translocate to the nucleus, where it binds to gene promoters (e.g., IL-1β, IL-6, TNF-α), initiating transcription. Pro-inflammatory cytokines further stimulate NF-κB, creating a positive feedback loop.[8] We hypothesize that *Sphingomonas mucosissima* metabolites lower ROS levels post-UVB exposure, thereby inhibiting ROS-activated NF-κB, reducing inflammation, and protecting cells.

Future research will focus on two key fields: identifying specific active components in the *Sphingomonas mucosissima* metabolite mixture through mass spectrometry and examining upstream signaling proteins affecting the NF-κB pathway to determine if other mechanisms, aside from ROS reduction, are involved in inhibiting NF-κB signaling by these metabolites.

**References**

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