Scalp bacterial species influence SIRT1 and TERT expression in keratinocytes

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Regular paper

Title: Scalp bacterial species influence *SIRT1* and *TERT* expression in keratinocytes

Short title: Scalp bacteria impact keratinocyte *SIRT1*/*TERT*

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ABSTRACT

Scalp bacteria on the human scalp and scalp hair comprise distinct community structures for sites and individuals. To evaluate their effect on human keratinocyte cellular activity, including that of the hair follicular keratinocytes, the expression of several longevity genes was examined using HaCaT cells. A screening system that uses enhanced green fluorescent protein (EGFP) fluorescence was established to identify scalp bacteria that enhance silent mating type information regulation 2 homolog-1 (*SIRT1*) promoter activity in transformed HaCaT cells (SIRT1p-EGFP). The results of quantitative polymerase chain reaction (qPCR) revealed that several predominant scalp bacteria enhanced (*Cutibacterium acnes* and *Pseudomonas lini*) and repressed (*Staphylococcus epidermidis*) the expressions of *SIRT1* and telomerase reverse transcriptase (*TERT)* genes in HaCaT cells. These results suggest that the predominant scalp bacteria are related to the health of the scalp and hair, including repair of the damaged scalp and hair growth, by regulating gene expression in keratinocytes.

Keywords: Scalp bacteria, *SIRT1*, *TERT*, HaCaT cell, keratinocyte

Parts of the human body, including the scalp, hair, skin, and gut, is a habitat for many microorganisms such as bacteria (Scharschmidt *et al.* 2013; Nakayama *et al.* 2015; Watanabe *et al.* 2020). Bacterial community structures in the human body have been elucidated in many studies, and they have been reported to be distinct depending on the body sites and individuals (Grice *et al.* 2011; Byrd *et al.* 2018). In addition, indigenous bacteria in the human skin or intestine continuously acquire essential nutrients from their hosts (Byrd *et al.* 2018). However, several indigenous bacteria are known for their positive and negative effects to the hosts, including an antimicrobial barrier by *Staphylococcus epidermidis* and excessive inflammation by *Staphylococcus aureus*, respectively (Byrd *et al.* 2018; Xu *et al.* 2016).

Human scalp hair consists of a shaft and root, and bacterial community structures on human scalp hair have recently been analyzed (Tridico *et al.* 2014; Nishi *et al.* 2017; Watanabe *et al.* 2019, 2020, 2021). Scanning electron microscopy (SEM) directly elucidated the presence of bacteria from the hair root to the tip of hair shafts and revealed higher bacterial cell densities of ~ 10^7 cells/cm^2 at the hair roots than ~ 10^5 cells/cm^2 at the hair shafts (Watanabe *et al.* 2019). Our previous studies on the analysis of bacterial community structure on human scalp hair indicated that bacteria on hair shafts originated from those on hair roots (Watanabe *et al.* 2019) and that bacterial community structures

differed among individuals with the Genera of *Cutibacterium*, *Pseudomonas*, and *Staphylococcus* being predominant (Watanabe *et al.* 2021). In addition, external factors, such as hair-care treatments, regardless of gender, are considered to contribute to a distinct bacterial community structure in the hair shafts of each individual (Watanabe *et al.* 2021). Furthermore, these genera are also predominant on the human scalp, although their relative abundances on the human scalp differ from those in human scalp hair (Watanabe *et al.* 2020). The functions of the bacterial community on the human scalp and scalp hair (scalp bacteria) are not well understood.

Several epidemiological studies have indirectly reported correlations between scalp bacteria and dermatosis (Pinto *et al*. 2019; Filaire *et al.* 2020). Pinto et al. (2019) and Filaire et al. (2020) compared bacterial community structures on the human scalp between healthy individuals and patients with alopecia areata and male pattern alopecia and suggested that the bacterial community structure on the scalp is a causal factor of scalp dermatosis (Pinto *et al.* 2019; Filaire *et al*. 2020). However, the mechanism underlying bacteria-induced scalp dermatosis has not yet been elucidated. To the best of our knowledge, there are few reports on the correlation between scalp bacteria and hair follicle cells; nevertheless, scalp and scalp hair conditions are affected by scalp cell activity.

Bacterial cells exist at a high density of 10^7 cells/cm^2 on the scalp hair root (Watanabe *et al.* 2019), while in contact with human cells in hair follicles. We hypothesized that these scalp bacteria would interact with human keratinocytes, including hair follicles, and possibly change cellular activities, such as the hair growth cycle (Stenn *et al.* 2001). Thus, it is important to elucidate how scalp bacteria affect human cells in the hair follicles of keratinocytes to deeply understand the exact hair and scalp conditions.

Because aging in the hair follicles of keratinocytes is reported to lead to decreases in hair diameter, hair density, and hair pigmentation (Turner *et al.* 2016), a longevity gene with anti-aging effect including ~~T~~the silent mating type information regulation 2 homolog-1 (*SIRT1*) (Lee *et al.* 2016) would be related to cellular activities in the hair follicles of keratinocytes. In addition, telomerase reverse transcriptase (*TERT*) in hair follicle of keratinocytes is considered to play a role in hair growth (Sarin *et al.* 2005; Kubo *et al.* 2020). Therefore, the aim of this study was to examine the effect of predominant scalp bacteria on the expression of several longevity genes, including ~~silent mating type information regulation 2 homolog-1 (~~*SIRT1*~~)~~ and ~~telomerase reverse transcriptase (~~*TERT*~~)~~, which are related to systemic antiaging and hair growth promotion, respectively, in keratinocytes.

Materials and methods

Used predominant scalp bacteria and bacterial culture

Species of predominant scalp bacteria (Watanabe *et al.* 2019) were used, as shown in Table 1. Some species were isolated from the scalp and scalp hair in our laboratory (unpublished data), while the others were obtained from Biological Resource Center, NITE (NBRC), Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM), and Laboratory of Microbiology, Department of Biochemistry and Microbiology, Faculty of Sciences of Ghent University (LMG). Each bacterial species was cultured for 1-4 days under the appropriate medium, temperature, and atmospheric conditions (Table S1). The compositions of the respective media were: nutrient broth with 5.0 g/L of peptone (Becton, Dickinson and Company (BD), NJ, USA) and 3 g/L of meat extract (Nacalai Tesque, Kyoto, Japan); LB with 1 g/L of tryptone (Thermo Fisher Scientific, Tokyo, Japan), 5 g/L of yeast extract (BD), and 10 g/L of NaCl; TSYB with 30 g/L of trypticase soy broth (BD) and 3 g/L of yeast extract (BD); NA with $MnSO_4$ and 5 g/L of peptone (BD), 3 g/L of meat extract (Nacalai Tesque), and 10 mg/L of $MnSO_4 \cdot 7H_2O$; 802 medium in NBRC with 1 g/L of Hipolypepton (Fujifilm Wako Pure Chemical CO., Osaka, Japan), 2 g/L of yeast extract (BD), and 1 g/L of $MgSO_4 \cdot 7H_2O$; Peptone Yeast Extract Alkaline with 8 g/L of peptone (BD), 3 g/L of yeast extract (BD), 1 g/L of K_2HPO_4, 3.5 mg/L of EDTA-

2Na, 3 mg/L of $ZnSO_4 \cdot 7H_2O$, 10 mg/L of $FeSO_4 \cdot 7H_2O$, 2 mg/L of $MnSO_4 \cdot 5H_2O$, 1 mg/L of $CuSO_4 \cdot 5H_2O$, 2 mg/L of $Co(NO_3)_2 \cdot 6H_2O$, and 1 mg/L of H_3BO_3; CASO with 8 g/L of tryptone (BD), 15 g/L of yeast extract (BD), 5.0 g /L of peptone from soymeal (Nacalai Tesque), and 5 g/L of NaCl; and GAM with 59 g/L of GAM Broth (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). An AnaeroPack (Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan) was used in the anaerobic jar for anaerobic cultivation. Cultured bacterial cells were washed three times with 2.24×10^{-2} M phosphate-buffered saline (PBS) buffer containing 137 mM NaCl. The suspension was heated at 100 °C for 30 min to inactivate the bacterial cells and was subsequently freeze-dried. The freeze-dried bacteria were resuspended in PBS when the number of bacteria reached 3,000 μg-dried cells/ml for further use and cultivation with human cell.

Human cell culture

The HaCaT cell (CLS Cell Line Service GmbH, Eppelheim, Germany) derived from a human keratinocyte cell line was cultured in Dulbecco's Modified Eagle's Medium (DMEM; Nissui Pharmaceutical Co., Ltd.) supplemented with 10% fetal bovine serum (FBS; Life Technologies, Gaithersburg, MD, USA) at 37 °C under 5% CO_2 atmosphere. In a screening system for scalp bacteria activating the *SIRT1* gene expression, HaCaT

cells transformed with the enhanced green fluorescent protein (EGFP) expression vector under the control of human *SIRT1* promoter (HaCaT (SIRT1p-EGFP)) (Harada *et al.* 2016) were used. The cells in DMEM with 10% FBS were cultured using a 96-well plate with a 100-µl working volume per well that included 1 µl or 10 µl of 3,000 µg-dried cells/ml scalp bacterial cell suspensions (final conc. 30 µg- or 300 µg-dried cells/mL), 1 µl or 10 µl of PBS (negative control for scalp bacteria), 1 µl of 1 mM resveratrol in dimethyl sulfoxide (DMSO) (final conc. 10 µM, positive control) (Lee *et al.* 2019), and 1 µl of DMSO (negative control for resveratrol) for 48 h at 37 °C under 5% CO_2 atmosphere. The intensity at an emission wavelength of 507 nm derived from pSIRT1p-EGFP was monitored using the IN Cell Analyzer 2200 (GE Healthcare, Little Chalfont, UK) at an excitation wavelength of 488 nm (Chong *et al.* 2019).

Quantitative reverse transcription-polymerase chain reaction

HaCaT cell (non-transformed) were cultured with scalp bacterial cell suspension (30 µg-dried cells per well), PBS (negative control for scalp bacteria), 10 µM resveratrol in DMSO (positive control) (Lee *et al.* 2019), or DMSO (negative control for resveratrol) for 48 h. The total RNA of HaCaT cell was extracted using the High Pure RNA Isolation kit (Roche Diagnostics, Manheim, Germany) and was reverse-transcribed into cDNA

using RevrerTra Ace (Toyobo, Osaka, Japan). Quantitative polymerase chain reaction (qPCR) was performed in triplicate using the Thunderbird SYBR qPCR mix (Toyobo) in a Thermal Cycler Dice Real Time System TP-800 (Takara, Shiga, Japan). The samples were tested in triplicate at least, and *SIRT1* and *TERT* expression levels were normalized to the corresponding β-actin expression level (Chong *et al.* 2019). The following qPCR primer sets were used. β-actin: forward, 5′ -TGGCACCCAGCACAATGAA-3′ and reverse, 5 ′ - CTAAGTCATAGTCCGCCTAGAAGC-3′; *SIRT1*: forward, 5′-GCCTCACATGCAAGCTCTAGTGAC-3′ and reverse, 5′-TTCGAGGATCTGTGCCAATCATAA-3′; *TERT*: forward, 5′-CGTACAGGTTTCACGCATGTG-3′ and reverse, 5′-ATGACGCGCAGGAAAAATG-3′ (Harada *et al.* 2016; Kubo *et al.* 2020). The qPCR condition was as follows: 45 cycles of 3-step PCR (95 °C for 5 sec, 60 °C for 10 sec, and 72 °C for 20 sec), and the final extension at 60 °C for 30 sec.

Statistical analysis

All experiments were performed at least three times, and the corresponding data are shown below. Results are presented as mean ± standard deviation. Statistical significance was determined using the two-sided Student's t-test. Statistical significance was defined

as *P <0.05.

Results

Establishment of a screening system for scalp bacteria that enhance *SIRT1* promoter activity in HaCaT cells

To elucidate the effect of scalp bacteria on the cellular activities of keratinocytes, we first established a rapid screening system using HaCaT (SIRT1p-EGFP) cells, derived from a human keratinocyte cell line, transformed with an EGFP expression vector under the control of the human *SIRT1* promoter. HaCaT (SIRT1p-EGFP) cells were cultured with 30 or 300 μg/mL of five representative scalp bacteria (*Cutibacterium acnes*, *Staphylococcus epidermidis*, *Pseudomonas lini*, *Moraxella oslorensis*, and *Delftia acidovorans*) for 48 h, and the intensity of EGFP fluorescence was measured as the *SIRT1* promoter activity (Figure 1). At a concentration of 300 μg/mL per well, the fluorescence intensities increased for all bacteria, which made it difficult to distinguish or evaluate the effects of the scalp bacterial species on cellular activities. In contrast, a few scalp bacteria, including *Pseudomonas lini* ~~*Staphylococcus epidermidis*~~ and *Delftia acidovorans* significantly enhanced fluorescence intensity at 30 μg/mL per well. Therefore, scalp bacteria were added to HaCaT cells at a concentration of 30 μg/mL for subsequent experiments.

Screening of scalp bacteria that enhance *SIRT1* promoter activity in HaCaT (SIRT1p-EGFP) cells

The scalp bacteria enhancing *SIRT1* promoter activity in human keratinocytes of HaCaT (SIRT1p-EGFP) cells were screened by the system established, as described in the previous section, using 30 μg/mL per well with 24 species (Actinobacteria, 8 species; Firmicutes, 4 species; Proteobacteria, 12 species) (Figure 2). From four independent experiments, the relative EGFP fluorescence was significantly elevated in HaCaT cells (SIRT1p-EGFP) cultured with 13 bacterial species that were comparable to the results obtained from the experiment with positive control using resveratrol as follows: five belonging to Actinobacteria, namely, *Brachybacterium muris*, *Corynebacterium lipophiloflavum*, *Dermacoccus nishinomiyaensis*, *Rothia kristinae*, and *Micrococcus luteus*; ~~one belonging to Firmicutes, namely, *Staphylococcus argenteus*; and~~ eight~~seven~~ belonging to Proteobacteria, namely, *Pseudomonas lini*, *Pseudomonas alcaliphila*, *Pseudomonas lundensis*, *Pseudomonas juntendi*, *Moraxella osloensis*, *Delftia acidovorans*, *Methylobacterium longum,* and *Roseomonas mucosa*. In contrast, the other 11 species did not show any significant increase in the relative EGFP fluorescence. These results demonstrate that the enhancement of the relative EGFP fluorescence was dependent on the scalp bacterial species in the established system and that 13 scalp

bacteria, including the predominant species, activated the *SIRT1* promoter in HaCaT (SIRT1p-EGFP) cells.

Evaluation of human endogenous *SIRT1* gene expression in non-transformed HaCaT cells

The *SIRT1* gene that encodes silent mating-type information regulation 2 homolog-1 is involved in regulating cellular senescence and aging (Chen *et al.* 2020). We proceeded to determine the effect of *SIRT1* gene expression in HaCaT (non-transformed) cells by reverse transcription PCR (RT-PCR) by activation of the *SIRT1* promoter using the 13 species described in the previous section and five predominant scalp bacterial species (*Curibacterium acnes, Staphylococcus epidermidis, Staphylococcus argenteus, Pseudomonas antarctica, Pseudomonas coleopterorum,* and *Moraxella osloensis*) with >4% relative abundances, as reported previously (Watanabe *et al.* 2021) (Figure 3). The results showed that 13 scalp bacterial species belonging to two phyla and eight genera significantly promoted *SIRT1* expression (*P <0.05), namely, *Cutibacterium acnes*, *Corynebacterium lipophiloflavum*, *Dermacoccus nishinomiyaensis*, and *Micrococcus luteus* belonging to Actinobacteria and *Pseudomonas lini*, *Pseudomonas alcaliphyla*, *Pseudomonas antarctica*, *Pseudomonas coleoperorum*, *Pseudomonas lumdensis*,

Pseudomonas juntendi, *Moraxella osloensis*, *Delftia acidovorans*, and *Roseomonas mucosa* belonging to Proteobacteria. In contrast, *Staphylococcus epidermidis* and *Staphylococcus argenteus* belonging to Firmicutes repressed *SIRT1* expression. ~~The relative EGFP fluorescence as an indicator of *SIRT1* promoter activity in HaCaT (SIRT1p-EGFP) cells was not consistent with *SIRT1* gene expression, possibly due to cell toxicity.~~ Among the predominant scalp bacteria with >1% relative abundance, the four genera *Cutibacterium*, *Pseudomonas*, *Delftia*, and *Moraxella* significantly enhanced *SIRT1* expression in non-transformed HaCaT cells. Scalp bacterial species present in minority (*Corynebacterium, Dermacoccus, Micrococcus* and *Rosemonas*) with a relative abundance <0.5% also promoted *SIRT1* expression in non-transformed HaCaT cells.

Evaluation of human endogenous *TERT* gene expression in non-transformed HaCaT cells

TERT gene encoding telomerase reverse transcriptase is induced by activated *SIRT1* (Huang *et al.* 2008; Yamashita *et al.* 2012), and is considered to enhance hair growth, trigger the initiation of a new phase of hair follicle growth, and promote hair synthesis (Herrera *et al.* 1999; Sarin *et al.* 2005; Zhang *et al.* 2014; Kubo *et al.* 2020). *TERT* expression by the predominant scalp bacterial species that significantly increased *SIRT1*

expression was evaluated by RT-PCR in non-transformed HaCaT cells (Figure 4). The results showed that eight scalp bacterial species belonging to two phyla (Actinobacteria and Proteobacteria) significantly increased *TERT* expression as follows: Actinobacteria, *Cutibacterium acnes* and *Micrococcus luteus*; Proteobacteria, *Pseudomonas lini*, *Pseudomonas alcaliphilia*, *Pseudomonas antarctica, Pseudomonas coleopterorum, Pseudomonas lundensis, and Delftia acidovorans*. In contrast, *Moraxella osloensis* and *Staphylococcus argenteus*, which belong to the phyla Proteobacteria and Firmicutes, respectively, significantly suppressed *TERT* expression. These results indicate that the predominant scalp bacterial species would promote the expression of *TERT* along with that of *SIRT1*.

Discussion

Scalp bacteria have been reported to exist not only on the human scalp but also on human scalp hair (the root and shaft) with unique bacterial community structures depending on the host (Watanabe *et al*. 2019; 2020; Nishi *et al*. 2017). Based on the high cell density of scalp bacteria on human scalp at 10^7 cells/cm^2, scalp bacteria can obtain nutrients from human cells as hosts for proliferation (Watanabe *et al.* 2019). Although epidemiological surveys suggest that the scalp bacterial community plays a role in scalp

dermatosis involving human cells in hair follicles, little is known about the role of scalp bacteria in keratinocytes, including the cells of hair follicles (Pinto *et al.* 2019; Filaire *et al.* 2020). Our study provided the first direct insight into the changes in gene expression in keratinocytes induced by scalp bacteria at the cellular level. In this section, we discuss each point with the focus on the two genes of interest (*SIRT1* and *TERT*) that are affected by scalp bacteria.

The roles of keratinocyte *SIRT1* in the repair of DNA damage by UV-B and in the enhancement of diminished mitochondrial activity that would lead to hair loss from incompetent follicles (Nicu *et al.* 2020) have been reported (Wood 1999; Ming *et al.* 2010; Tang 2016; Jarrett *et al.* 2018; Chong *et al.* 2019). The predominant scalp bacteria with >7% relative abundance, including *Cutibacterium, Pseudomonas,* and *Staphylococcus* act on *SIRT1* expression in keratinocytes (Figure 3). Although several types of plants (ex. grape ~~, seaberry,~~ and Pomegranate ~~garlic~~) and mushrooms (ex. *Trametes versicolor* and *Tremella fuciformis* ~~snow fungus~~) have been reported to enhance human *SIRT1* expression *in vitro* (Kim 2016; Shen *et al.* 2017; Chong *et al.* 2018, 2019; Song *et al.* 2018; Lorz *et al.* 2019; Choi *et al.* 2020; Zhang *et al.* 2020; Kubo *et al.* 2020; Letsiou *et al.* 2020; Niimi *et al.* 2021). Among them, only polyphenol derivatives from plants have been elucidated as the *SIRT1* enhancers (Chong *et al.* 2019; Kubo *et al.* 2020),

and the scalp bacterial samples used in this study would not contain polyphenols. Because dead cells of scalp bacteria were used by removing culture broth, it is suggested that not extracellular metabolites but cell structures or intracellular metabolites would stimulate *SIRT1* expression in keratinocytes. To the best of our knowledge, this is the first report of scalp bacteria enhancing *SIRT1* expression. Considering these studies and our results, it can be presumed that the predominant scalp bacteria control the normal hair cycle in some manner (Geyfman *et al.* 2010; Horimoto *et al.* 2017; Liu *et al.* 2018).

In contrast, *TERT* has been reported to activate stem cells in hair follicles in the telogen phase to enter the anagen phase, which leads to hair growth promotion (Herrera *et al.* 1999; Sarin *et al.* 2005; Kubo *et al.* 2020). In addition, precocious loss and graying of hair are attributed to *TERT* inactivation in the stem cells of hair follicles (Stone *et al.* 2021). Compared with *SIRT1*, a few studies have reported the enhancement of *TERT* expression in strawberries, apples, and seaberries *in vitro* (Kubo *et al.* 2020). In our study, the predominant scalp bacteria with >7% relative abundance, including *Cutibacterium acnes* and *Pseudomonas lini,* stimulated *TERT* expression in keratinocytes (Figure 4). Notably, these scalp bacteria also activated *SIRT1* expression (Figure 3). These results suggest that the predominant scalp bacteria contribute to hair growth and prevention of hair loss and graying by activating *TERT* in the follicular stem cells.

In this study, three patterns of significant changes in *SIRT1* and *TERT* expression were observed (Figures 3 and 4): (I) Activation and Activation, by *Cutibacterium acnes, Micrococcus lutesu*, *Pseudomonas lini*, *Pseusdomonas alcaliphila*, *Pseudomonas coleopterorum*, and *Delftia acidovorans*; (II) Activation and Repression, by *Moraxella osloensis*, and (III) Repression and Repression, by *Staphylococcus argenteus* (Figures 3 and 4), respectively for *SIRT1* and *TERT*. PPAR (peroxisome proliferators-activated receptor) is considered to be activator for enhancement of SIRT 1 expression by (Shen *et al.* 2016), corresponding to the patterns I and II. ~~*SIRT1* expression is repressed by repressors such as p53 and FOXO3a (Yang *et al.* 2022), which would contribute to the regulation of *SIRT1* expression by scalp bacteria (Figure 3).~~ Enhancement of *TERT* expression associated with *SIRT1* expression is a well-known mechanism; *SIRT1* induces the formation of activators such as the bHLH transcription factor (c-MYC), which upregulates *TERT* (Choi *et al.* 2008; Yamashita *et al.* 2014), corresponding to the pattern I. On the other hand, *TERT* is repressed by repressors such as p21 (Colebatch *et al.* 2019), which downregulates *TERT*, similar to that seen in patterns II and III. *SIRT1* expression is repressed by repressors such as p53 and FOXO3a (Yang *et al.* 2022), which would contribute to the regulation of *SIRT1* expression by scalp bacteria ~~(Figure 3).~~ However, elucidation of the regulated mechanisms in keratinocytes by scalp bacteria is required for

developing beneficial applications of scalp bacteria for the health of the scalp and hair. Hence, further research is planned to uncover these mechanisms based on the categorization of scalp bacteria into the three patterns.

The predominant species on the human scalp, *Cutibacterium acnes* (412%) and *Pseudomonas lini* (8.819%) (Watanabe *et al.* 2019, 2020, 2021), simultaneously enhanced of *SIRT1* and *TERT* expressions (pattern I) in keratinocytes (Figures 3 and 4). Notably, these bacteria, when inhabiting the keratinocytes, are expected to contribute to antiaging actions in scalp cells (Ming *et al.* 2010; Chong *et al.* 2019) and hair restoration (Sahin *et al.* 2011; Kubo *et al.* 2020). *Cutibacterium acnes* has also been reported to have several functions as a resident bacterium on mammalian skin as follows (Tsuda *et al.* 2011; Shu *et al.* 2013): maintenance of the acidic layer, antimicrobial activity against pathogenic bacteria, and antitumor activity. Although *Pseudomonas lini* has been reported to exist in the neonatal gut (Dobbler *et al.* 2019), its function in the skin and scalp is not yet understood. Future studies must be aimed at investigating the relationship between hair and scalp disorders and the predominant scalp bacteria. These fundamentals will be essential for developing a new scalp-related tonic containing valuable scalp bacteria.

Conclusion

First, we provided direct insights into the effects of scalp bacteria on the host. Several predominant scalp bacterial species contribute to the regulation of *SIRT1*, which regulates antiaging activity, and *TERT*, which is related to hair growth in keratinocytes. Scalp bacteria inhabiting hair follicles have the potential to modulate gene expression in these cells on a routine basis. This study suggests that the predominant scalp bacteria, particularly *Cutibacterium acnes* and *Pseudomonas lini*, contribute to the homeostatic growth of hair within the hair follicles.

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Supplementary material

Supplementary material is available at *Bioscience, Biotechnology, and Biochemistry* online.

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

Author's contribution

Azusa Yamada performed experiments, analyzed data, prepared figures, and wrote the manuscript. Kota Watanabe and Yuri Nishi provided the scalp bacterial data and helped with data analysis. Yoshinori Katakura designed experiments, helped with data analysis, provided technical expertise, and edited the article. Yukihiro Tashiro, Mugihito Oshiro and Kenji Sakai supervised the project from experimental design to submission of the manuscript. All authors agreed and approved the manuscript to be published.

Conflict of Interest

No potential conflict of interest was reported by the authors.

References

Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. *Nat Rev Microbiol* 2018;16:143–155.

Chen C, Zhou M, Ge Y *et al.* SIRT1 and aging related signaling pathways. *Mech Ageing Dev* 2020;187:111215.

Choi HJ, Alam MB, Baek ME *et al.* Protection against UVB-induced photoaging by *Nypa fruticans* via inhibition of MAPK/AP-1/MMP-1 signaling. *Oxid Med Cell Longev* 2020;2020:1–14.

Choi J, Southworth LK, Sarin KY *et al.* TERT promotes epithelial proliferation through transcriptional control of a Myc- and Wnt-related developmental program. *PLoS Genet* 2008;4:0124–0138.

Chong Z, Matsuo H, Kuroda M *et al.* Mushroom extract inhibits ultraviolet B-induced cellular senescence in human keratinocytes. *Cytotechnology* 2018;70:1001–1008.

Chong Z, Matsuo H, Onoue S *et al.* Identification of polyphenols that repair the ultraviolet-B-induced DNA damage via SIRT1-dependent XPC/XPA activation. *J Funct Foods* 2019;54:119–127.

Colebatch AJ, Dobrovic A, Cooper WA. TERT gene: Its function and dysregulation in cancer. *J Clin Pathol* 2019;72:281–284.

Dobbler P, Mai V, Procianoy RS *et al.* The vaginal microbial communities of healthy expectant Brazilian mothers and its correlation with the newborn's gut colonization. *World J Microbiol Biotechnol* 2019;35:1–14.

Geyfman M, Andersen B. Clock genes, hair growth and aging. *Aging (Albany NY)* 2010;2:122–128.

Grice EA, Segre JA. The skin microbiome. *Nat Rev Microbiol* 2011;9:244–253.

Harada G, Pattarawat P, Ito K *et al. Lactobacillus brevis* T2102 suppresses the growth of colorectal cancer cells by activating SIRT1. *J Funct Foods* 2016;23:444–452.

Herrera E, Samper E, Martín-Caballero J *et al.* Disease states associated with telomerase deficiency appear earlier in mice with short telomeres. *EMBO J* 1999;18:2950–2960.

Horimoto K, Kunimoto R, Hashimoto R *et al.* SIRT1 negatively regulates the expression of Prl2C3, a senescence-associated protein. *Sapporo Med J* 2017;86:63–71.

Huang J, Gan Q, Han L *et al.* SIRT1 overexpression antagonizes cellular senescence with activated ERK/S6k1 signaling in human diploid fibroblasts. *PLoS One* 2008;3.

Jarrett SG, Carter KM, Bautista RM *et al.* Sirtuin 1-mediated deacetylation of XPA DNA repair protein enhances its interaction with ATR protein and promotes cAMP-induced DNA repair of UV damage. *J Biol Chem* 2018;293:19025–19037.

Kim HK. Protective effect of garlic on cellular senescence in UVB-exposed HaCaT human keratinocytes. *Nutrients* 2016;8.

Kubo C, Ogawa M, Uehara N *et al.* Fisetin promotes hair growth by augmenting TERT expression. *Front Cell Dev Biol* 2020;8:1–13.

Lee SH, Lee JH, Lee HY *et al.* Sirtuin signaling in cellular senescence and aging. *BMB Rep* 2019;52:24–34.

Letsiou S, Kapazoglou A, Tsaftaris A. Transcriptional and epigenetic effects of *Vitis vinifera* L. leaf extract on UV-stressed human dermal fibroblasts. *Mol Biol Rep* 2020;47:5763–5772.

Liu J, Xu Y, Wu Q *et al.* Sirtuin-1 protects hair follicle stem cells from TNFα-mediated inflammatory stress via activating the MAPK-ERK-Mfn2 pathway. *Life Sci* 2018;212:213–224.

Lorz LR, Yoo BC, Kim MY *et al.* Anti-wrinkling and anti-melanogenic effect of *Pradosia mutisii* methanol extract. *Int J Mol Sci* 2019;20.

Ming M, Shea CR, Guo X *et al.* Regulation of global genome nucleotide excision

repair by SIRT1 through xeroderma pigmentosum C. *Proc Natl Acad Sci U S A* 2010;107:22623–22628.

Nakayama J, Watanabe K, Jiang J *et al.* Diversity in gut bacterial community of school-age children in Asia. *Sci Rep* 2015;5:1–12.

Nicu C, Wikramanayake TC, Paus R. Clues that mitochondria are involved in the hair cycle clock: MPZL3 regulates entry into and progression of murine hair follicle cycling. *Exp Dermatol* 2020;29:1243–1249.

Niimi Y, Pérez-Bello D, Ihara K *et al.* Omega-7 oil increases telomerase activity and accelerates healing of grafted burn and donor site wounds. *Sci Rep* 2021;11:1–14.

Nishi E, Watanabe K, Tashiro Y *et al.* Terminal restriction fragment length polymorphism profiling of bacterial flora derived from single human hair shafts can discriminate individuals. *Leg Med* 2017;25:75–82.

Sarin KY, Cheung P, Gilison D *et al.* Conditional telomerase induction causes proliferation of hair follicle stem cells. *Nature* 2005;436:1048–1052.

Scharschmidt TC and Fischbach MA. What lives on our skin: Ecology, genomics and therapeutic opportunities of the skin microbiome. *Drug Discov Today Dis Mech* 2013;10:e83–e89.

Shen T, Duan C, Chen B *et al. Tremella fuciformis* polysaccharide suppresses

hydrogen peroxide-triggered injury of human skin fibroblasts via upregulation of SIRT1. *Mol Med Rep* 2017;16:1340–1346.

Shu M, Wang Y, Yu J *et al.* Fermentation of *Propionibacterium acnes*, a commensal bacterium in the human skin microbiome, as skin probiotics against methicillin-resistant *Staphylococcus aureus*. *PLoS One* 2013;8.

Song IB, Gu H, Han HJ *et al.* Omega-7 inhibits inflammation and promotes collagen synthesis through SIRT1 activation. *Appl Biol Chem* 2018;61:433–439.

Stenn KS, Paus R. Controls of hair follicle cycling. *Physiol Rev* 2001;81:449–494.

Stone RC, Aviv A, Paus R. Telomere dynamics and telomerase in the biology of hair follicles and their stem cells as a model for aging research. *J Invest Dermatol* 2021;141:1031–1040.

Tang BL. Sirt1 and the mitochondria. *Mol Cells* 2016;39:87–95.

Tridico SR, Murray DC, Addison J *et al.* Metagenomic analyses of bacteria on human hairs: A qualitative assessment for applications in forensic science. *Investig Genet* 2014;5.

Tsuda K, Yamanaka K, Linan W *et al.* Intratumoral injection of *Propionibacterium acnes* suppresses malignant melanoma by enhancing Th1 immune responses. *PLoS One* 2011;6.

Watanabe K, Nishi E, Tashiro Y *et al.* Mode and structure of the bacterial community on human scalp hair. *Microbes Environ* 2019;34:252–259.

Watanabe K, Yamada A, Nishi Y *et al.* Relationship between the bacterial community structures on human hair and scalp. *Biosci Biotechnol Biochem* 2020;84:2585–2596.

Watanabe K, Yamada A, Nishi Y *et al.* Host factors that shape the bacterial community structure on scalp hair shaft. *Sci Rep* 2021;11:1–11.

Wood RD. DNA damage recognition during nucleotide excision repair in mammalian cells. *Biochimie* 1999;81:39–44.

Yamashita S, Ogawa K, Ikei T *et al.* SIRT1 prevents replicative senescence of normal human umbilical cord fibroblast through potentiating the transcription of human telomerase reverse transcriptase gene. *Biochem Biophys Res Commun* 2012;417:630–634.

Yamashita S, Ogawa K, Ikei T *et al.* FOXO3a potentiates hTERT gene expression by activating c-MYC and extends the replicative life-span of human fibroblast. *PLoS One* 2014;9:2–8.

Yang Y, Liu Y, Wang Y *et al.* Regulation of SIRT1 and its roles in inflammation. *Front Immunol* 2022;13:1–16.

1 Zhang P, He L, Zhang J *et al.* Preparation of novel berberine nano-colloids for
2 improving wound healing of diabetic rats by acting Sirt1/NF-κB pathway. *Colloids*
3 *Surfaces B Biointerfaces* 2020;187:110647.
4
5

Figure Captions

Figure 1. Relative EGFP fluorescence of SIRT1 promoter activity in HaCaT (SIRT1p-EGFP) cell cultivated with selected predominant scalp bacteria at 30 μg/ml and 300 μg/ml per well. a) treated scalp bacteria at 30 μg/ml. b) treated with scalp bacteria at 300 μg/ml. EGFP fluorescence of HaCaT (SIRT1p- EGFP) cell after 48 h cultivation were monitored by the imaging cytometer (IN Cell Analyzer 2200). Results are expressed as mean ± standard error of 3 replicates. Statistical significance was defined at *P < 0.05.

Figure 2. Relative EGFP fluorescence of SIRT1 promoter activity in HaCaT (SIRT1p-EGFP) cell cultivated with scalp bacteria at 30 μg/ml per well. EGFP fluorescence of HaCaT (SIRT1p- EGFP) cell after 48 h cultivation were monitored by the imaging cytometer (IN Cell Analyzer 2200). Results are expressed as mean ± standard error of 3 replicates. Statistical significance was defined at *P < 0.05.

Figure 3. Relative expression levels of SIRT1 by qRT-PCR in non-transformed HaCaT cells cultured with scalp bacteria at 30 μg/ml per well by standardizing expression of β-catenin. Results are expressed as mean ± standard error of 3 replicates. Statistical significance was defined as *P < 0.05. Relative abundances of each bacterium were

referred to our previous reports (Watanabe *et al.* 2020, 2021).

Figure 4. Relative expression levels of *TERT* by qRT-PCR in non-transformed HaCaT cells with predominant scalp bacteria at 30 μg/ml per well by standardizing expression of β-catenin. Results are expressed as mean ± standard error of 3 replicates. Statistical significance was defined as *P < 0.05. Relative abundances of each bacterium were referred to our previous reports (Watanabe *et al.* 2020, 2021).

Graphical abstract caption

Schematic sketch of *SIRT1* and *TERT* gene expression in hair follicle keratinocytes by scalp bacteria.

Table

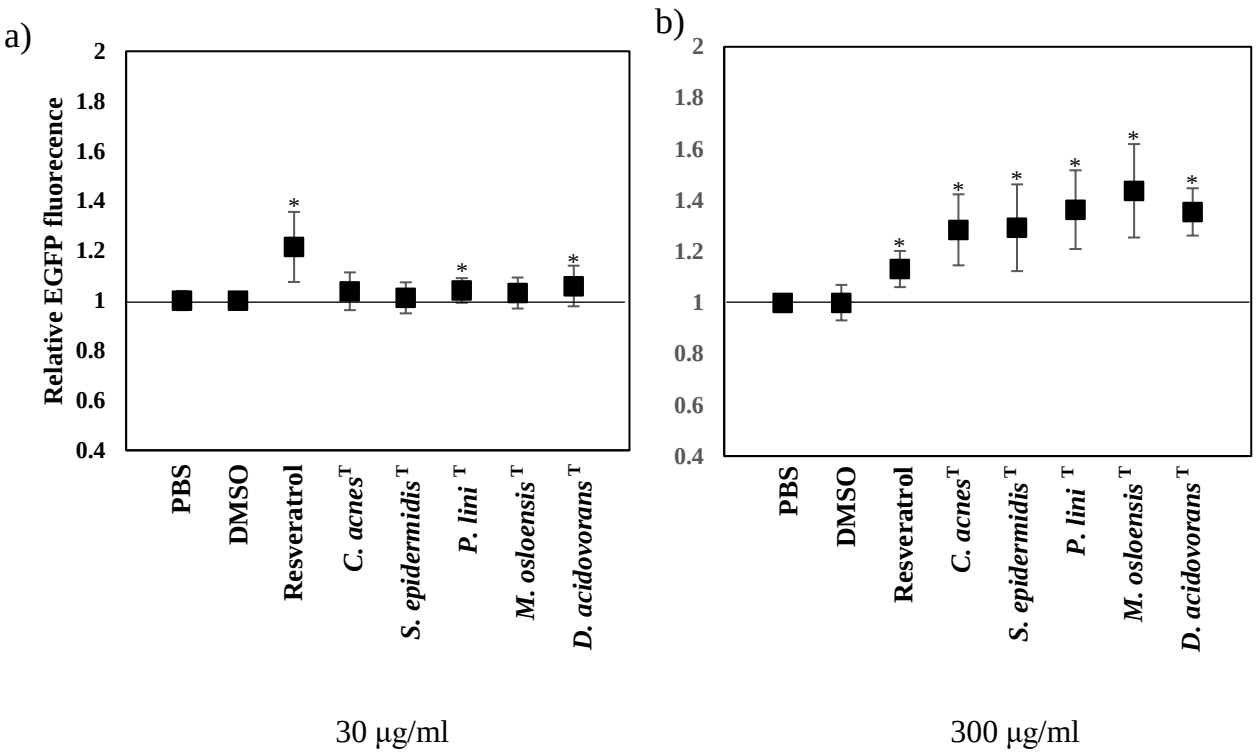
Table 1. The list of scalp bacteria used in experiments

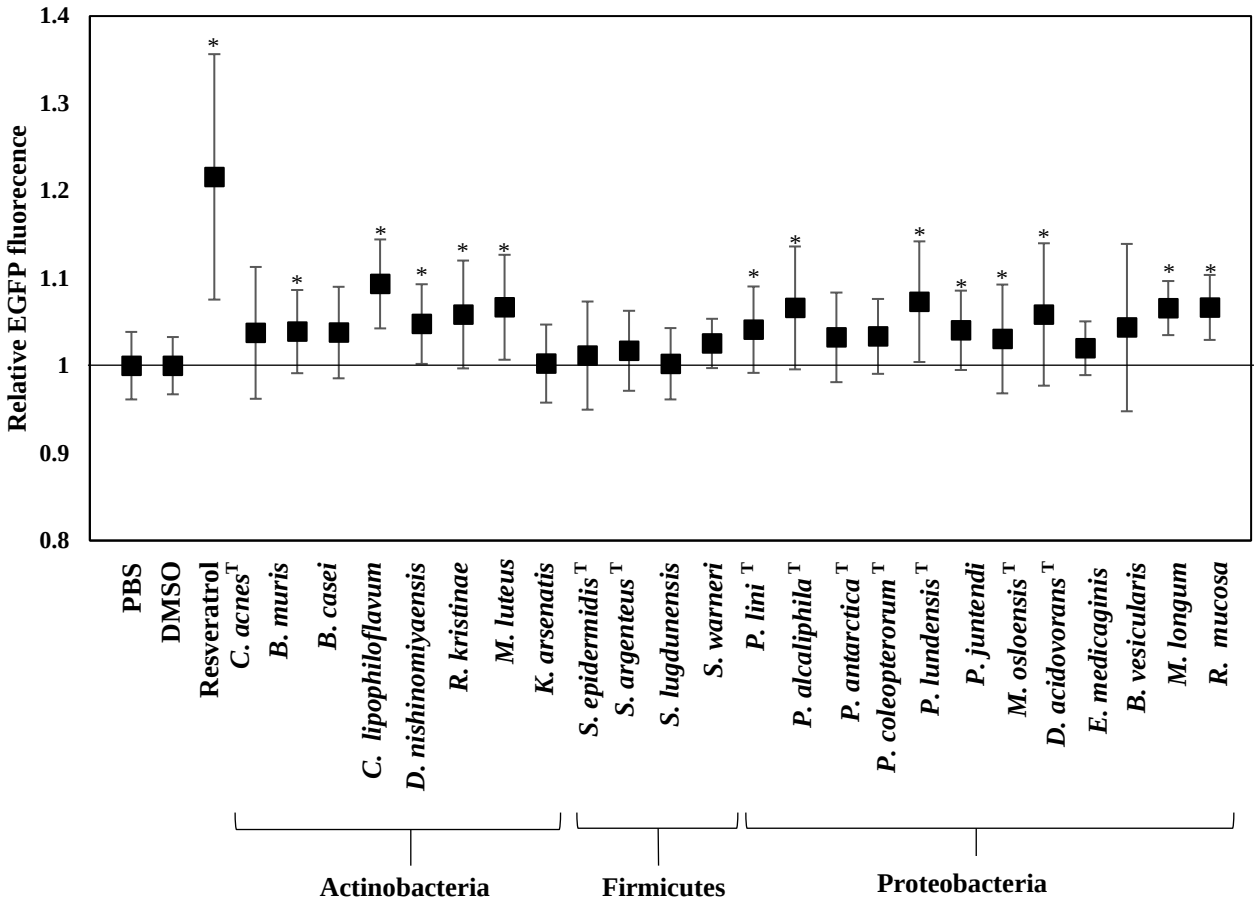
Phylum	Bacterial species	Origin	Abundance on human scalp hair (%) [a]
Actinobacteria	*Cutibacterium acnes*^{T}	NBRC107605	41.15
	Brachybacterium muris	IS[b]	<0.08
	Brevibacterium casei	IS[b]	<0.08
	Corynebacterium lipophiloflavum	IS[b]	0.23
	Dermacoccus nishinomiyaensis	IS[b]	0.09
	Rothia kristinae	IS[b]	<0.08
	Micrococcus luteus	IS[b]	0.65
	Kocuria arsenatis	IS[b]	<0.08
Firmicutes	*Staphylococcus epidermidis*^{T}	NBRC100911	7.24 (identical OTUs)[c]
	S. argenteus^{T}	DSM29875	
	S. lugdunensis	IS[b]	
	S. warneri	IS[b]	
Proteobacteria	*Pseudomonas lini*^{T}	DSM16768	8.75
	P. alcaliphila^{T}	NBRC102411	3.04
	P. antarctica^{T}	DSM15318	2.11
	P. coleopterorum^{T}	LMG28558	1.87
	P. lundensis^{T}	DSM6252	0.58
	P. juntendi	IS[b]	<0.08
	Moraxella osloensis^{T}	NBRC111460	4.45
	Delftia acidovorans^{T}	IS[b]	1.31
	Endobacter medicaginis	IS[b]	0.21
	Brevundimonas vesicularis	IS[b]	0.88
	Methylobacterium longum	IS[b]	0.12
	Roseomonas mucosa	IS[b]	<0.08

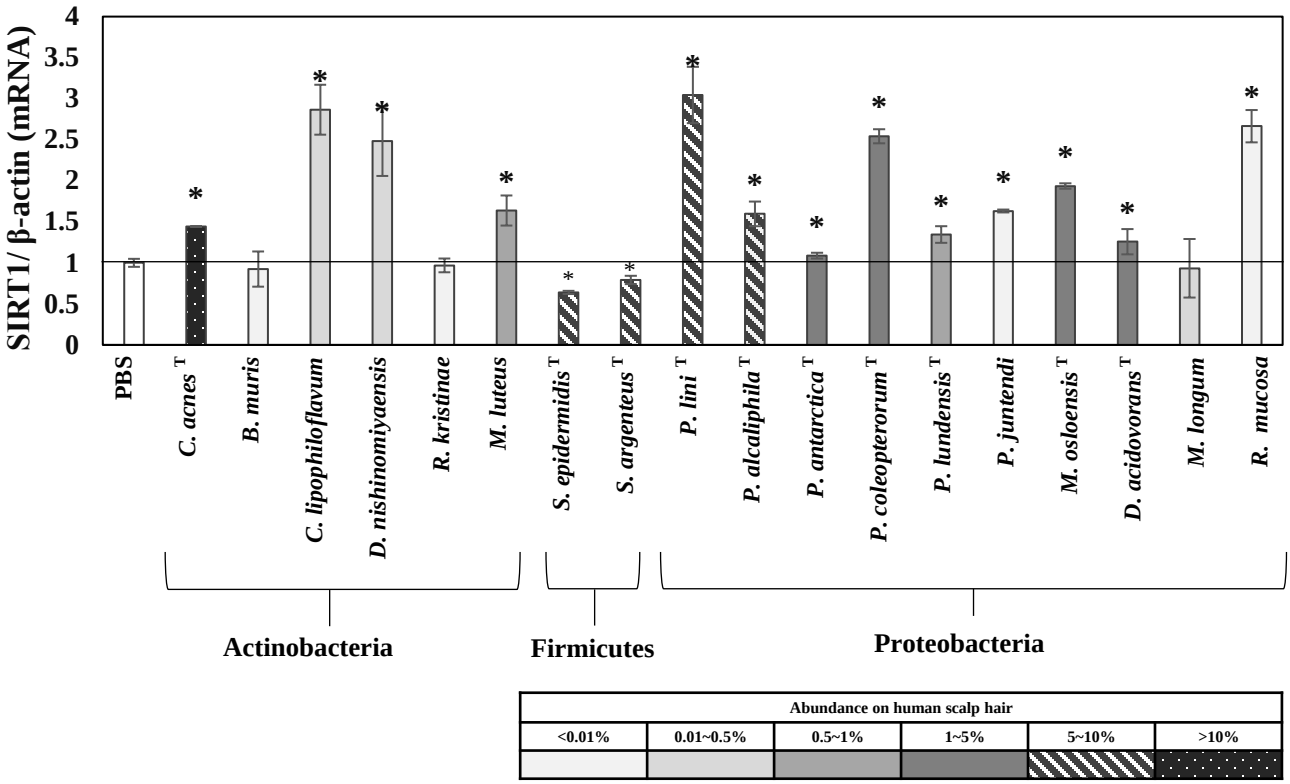
[a] Dominant abundance referred to the Watanabe(Watanabe *et al.* 2021).

[b] IS: isolate from human scalp hair

[c] OTUs: grouped into operational taxonomic units at >97% similarity (Watanabe *et al.* 2021)


a)
b)
Relative EGFP fluorecence
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
PBS
DMSO
Resveratrol
C. acnes T
S. epidermidis T
P. lini T
M. osloensis T
D. acidovorans T
30 μg/ml
300 μg/ml


Relative EGFP fluorecence
1.4
1.3
1.2
1.1
1
0.9
0.8
PBS
DMSO
Resveratrol
C. acnes T
B. muris
B. casei
C. lipophiloflavum
D. nishinomiyaensis
R. kristinae
M. luteus
K. arsenatis
S. epidermidis T
S. argenteus T
S. lugdunensis
S. warneri
P. lini T
P. alcaliphila T
P. antarctica T
P. coleopterorum T
P. lundensis T
P. juntendi
M. osloensis T
D. acidovorans T
E. medicaginis
B. vesicularis
M. longum
R. mucosa
Actinobacteria
Firmicutes
Proteobacteria


SIRT1/ β-actin (mRNA)
4
3.5
3
2.5
2
1.5
1
0.5
0
PBS
C. acnes T
B. muris
C. lipophiloflavum
D. nishinomiyaensis
R. kristinae
M. luteus
S. epidermidis T
S. argenteus T
P. lini T
P. alcaliphila T
P. antarctica T
P. coleopterorum T
P. lundensis T
P. juntendi
M. osloensis T
D. acidovorans T
M. longum
R. mucosa
Actinobacteria
Firmicutes
Proteobacteria
Abundance on human scalp hair
<0.01%
0.01~0.5%
0.5~1%
1~5%
5~10%
>10%

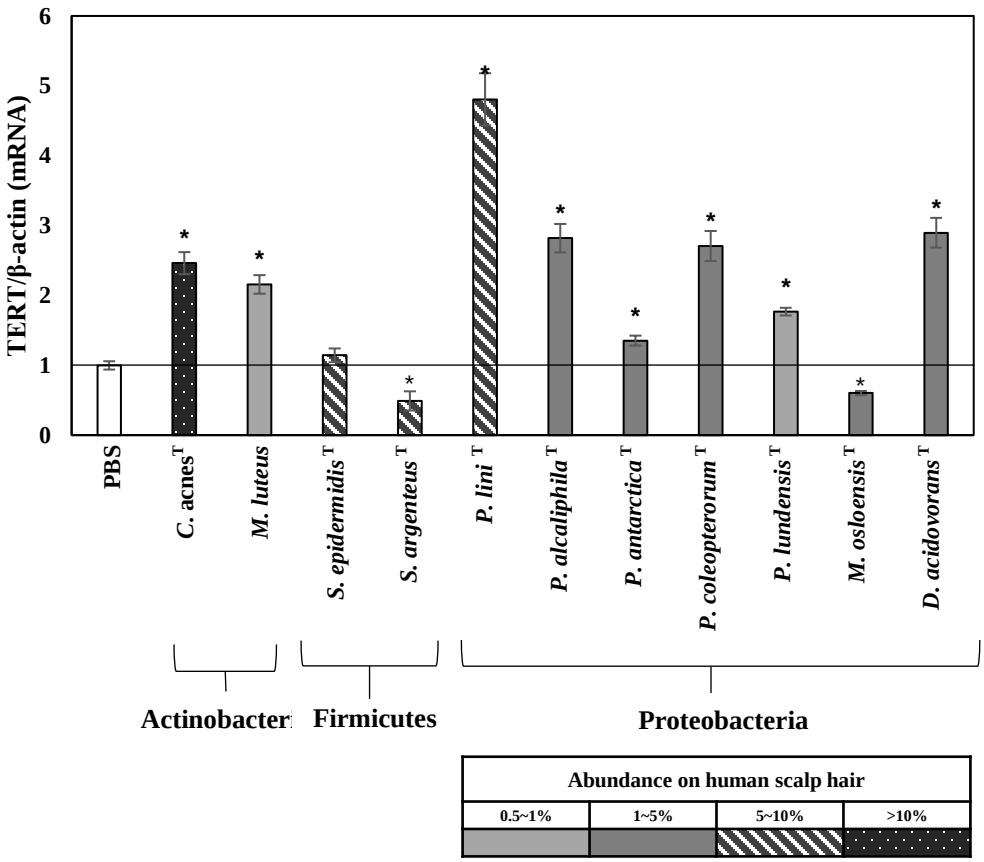
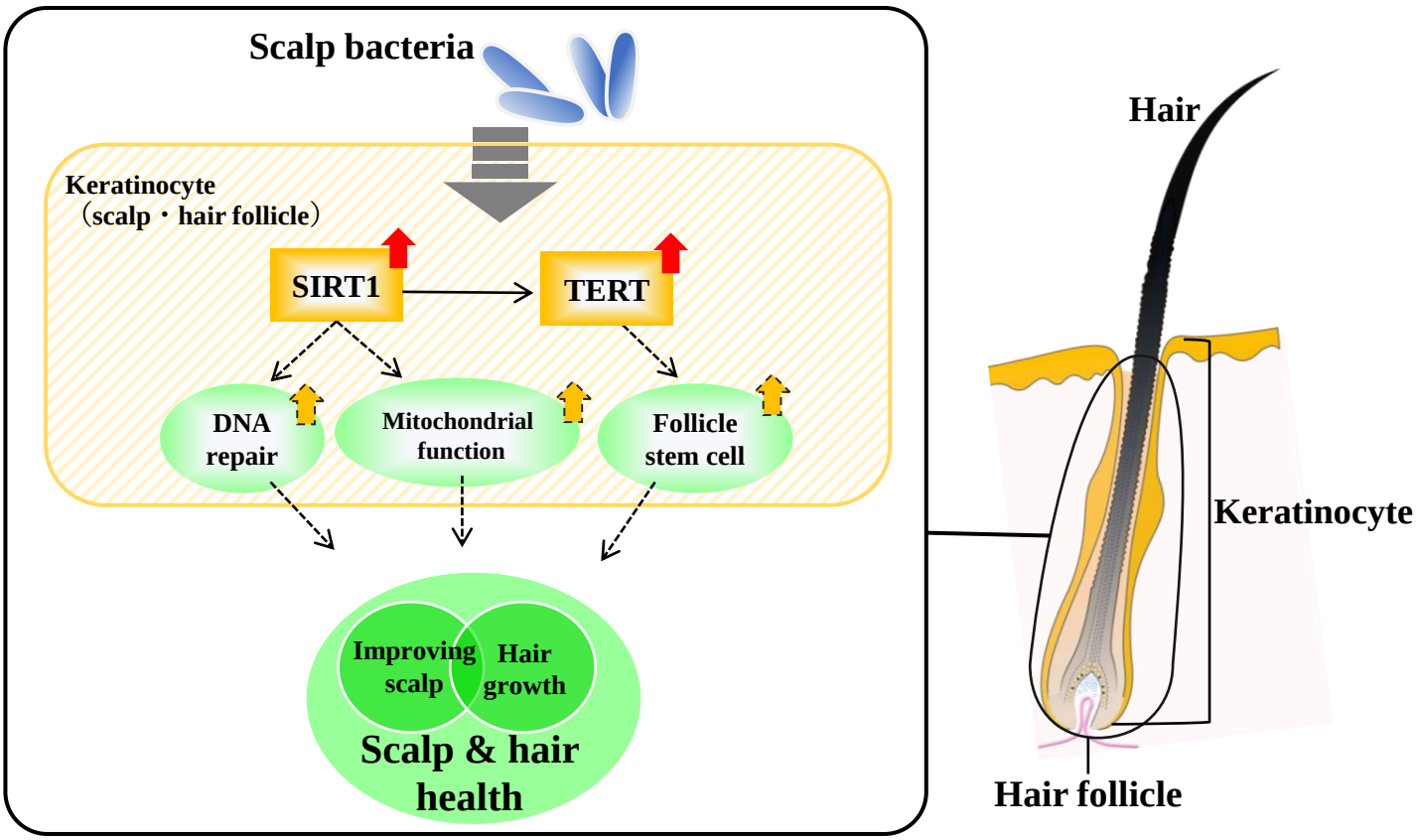

TERT/β-actin (mRNA)
0
1
2
3
4
5
6
PBS
C. acnes T
M. luteus
S. epidermidis T
S. argenteus T
P. lini T
P. alcaliphila T
P. antarctica T
P. coleopterorum T
P. lundensis T
M. osloensis T
D. acidovorans T
Actinobacteri
Firmicutes
Proteobacteria
Abundance on human scalp hair
0.5~1%
1~5%
5~10%
>10%

Table S1. Used scalp bacteria and culture condition

Phylum	Species	Medium	Cultivation temperature (°C)	Origin
Actinobacteria	*Cutibacterium acnes*^{T}	GAM	37	NBRC 107605
	Brachybacterium muris^{T}	TSY	30	IS[a]
	Brevibacterium casei^{T}	NA(m)	30	IS[a]
	Corynebacterium lipophiloflavum^{T}	TSY	30	IS[a]
	Dermacoccus nishinomiyaensis^{T}	NA	30	IS[a]
	Rothia kristinae^{T}	LB	30	IS[a]
	Micrococcus luteus^{T}	NA	30	IS[a]
	Kocuria arsenatis^{T}	NA	30	IS[a]
Firmicutes	*Staphylococcus epidermidis*^{T}	NBRC802	37	NBRC 100911
	Staphylococcus argenteus^{T}	COLUMBIA BLOOD MEDIUM	30	DSM 29875
	Staphylococcus lugdunensis^{T}	NA	30	IS[a]
	Staphylococcus warneri^{T}	LB	30	IS[a]
Proteobacteria	*Pseudomonas lini*^{T}	CASO	28	DSM 16768
	Pseudomonas alcaliphila^{T}	Peptone Yeast Extract Alkaline	25	NBRC 102411
	Pseudomonas antarctica^{T}	CASO	28	DSM 15318
	Pseudomonas coleopterorum^{T}	NA	26	LMG 28558
	Pseudomonas lundensis^{T}	NA	25	DSM 6252
	Pseudomonas juntendi^{T}	NA	30	IS[a]
	Moraxella osloensis^{T}	NBRC802	30	NBRC111460
	Delftia acidovorans^{T}	NBRC802	30	IS[a]
	Endobacter medicaginis^{T}	NA	30	IS[a]
	Brevundimonas vesicularis^{T}	LB	30	IS[a]
	Methylobacterium longum^{T}	NA	30	IS[a]
	Roseomonas mucosa^{T}	NA	30	IS[a]

[a] IS: isolate from human scalp hair


Scalp bacteria
Keratinocyte
（scalp・hair follicle）
SIRT1
TERT
DNA repair
Mitochondrial function
Follicle stem cell
Improving scalp
Hair growth
Scalp & hair health
Hair
Keratinocyte
Hair follicle