A 1,1′-biuracil from *Epidermidibacterium keratini* EPI-7 shows anti-aging effects on human dermal fibroblasts

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Introduction:

Human skin is composed of multiple layers including several cells types, fibers and lipids. In addition, the human skin is populated by diverse microorganisms such as fungi, viruses and bacteria which feed on sebum, lipids, and keratin from the skin [1]. Many studies that dramatic changes on the skin microbiome can alter skin conditions and cause diseases such as acne, psoriasis, and atopic dermatitis [2-4]. In this study, we collected skin-microbiome samples from two different age groups of females. One group was in their 20 s and the other was in their 40 s. Analysis of 16S rRNA gene sequences showed a newly found bacterium that shares 93.4% homology with the genus *Sporichthya*, indicating the discovery of a novel genus. We isolated a novel bacterial stain, *Epidermidibacterium keratini*, called EPI-7^T [5], from skin samples. Additionally, the younger skin appeared to have high proportion of EPI-7^T, while the older skin had no EPI-7^T but rather other types of bacteria. Skin microorganisms produce various metabolites and influence skin cells directly or indirectly [6]. Among these microorganisms, the most common are of the genus *Staphylococcus* and several species in the genus *Staphylococcus* have been linked to various inflammatory diseases of the skin; however, no study has linked these organisms to aging. In addition, no report has described the relationships between aging and skin microorganisms. Therefore, in the current study, we analyzed the distribution of EPI-7^T by age and investigated mechanisms related to aging.

Materials & Methods:

Epidermidibacterium keratini
EPI-7 in R2A medium was provided by COSMAX R&I Center and a voucher specimen (KHUNPCL-201805) has been deposited at the Laboratory of Natural Products Chemistry, Kyung Hee University.

Extraction and isolation
EPI-7^T culture solutions grown in R2A medium were centrifuged, filtrated, and evaporated under reduced pressure. The concentrates were extracted in 80% aqueous MeOH. The combined concentrates were poured into H_2O and successively extracted with EtOAc and n-BuOH. Each layer was concentrated under reduced pressure to obtain EtOAc (EPE), n-BuOH (EPB), and H_2O (EPH) fractions. Fraction EPE was applied to silica gel (SiO_2) column chromatography (c.c.) and eluted with EtOAc-n-BuOH–H_2O with monitoring by TLC to provide 12 fractions (EPE-1 to EPE-12). Fraction EPE-3 was subjected to octadecyl SiO_2 (ODS) c.c. to yield four fractions (EPE-3-1 to EPE-3-4) and a purified compound 1 (EPE-3-1). 1,1′-Biuracil (1) White amorphous powder; IR (KBr) vmax 1715, 1674, 1418 cm^{-1}; low resolution positive ESI/ MS *m/z* 113 $[M/2 + 1]^+$, 223 $[M + 1]^+$, 267 $[M + 2Na\text{-}H]^+$; High resolution positive ESI/MS *m/z* 223.0459 $[M + 1]^+$ (Calcd. for $C_8H_7N_4O_4$ 223.0467); ^{1}H- and ^{13}CNMR (600 and 150 MHz, DMSO-$_{d6}$, δ_H and δ_C).

Cell culture and treatment
The human fibroblast cell line (Hs68) were cultured in Dulbecco's modified Eagle's medium supplemented with 1% Antibiotic Antimycotic and 10% fetal bovine serum at 37 °C in an atmosphere of 5% CO_2. For UV irradiation and treatment, Hs68 cells were seeded at 80% confluence into 6-well plates and incubated in an atmosphere of 5% CO2 at 37 °C. After 24 h, the cells were washed once with phosphate-buffered saline (PBS) and placed in fresh PBS. Next, 12 mJ/cm^2 of UVB (wavelength 290–320 nm, maximum peak 311 nm) was applied in the presence of crosslinker (UVP; Upland, CA, USA), and then EPI-7 (0.1– 1%) or 1,1′-biuracil (0.1–10 ppm) was administered into the cells through serum-free medium for 24 h.

RNA isolation and real-time PCR
Total RNA was isolated from cells using TRIzol reagent according to the manufacturer's instruction. cDNA was synthesized from 1 μg of total RNA using Reverse Transcription Premix and gene expression signals were quantified with real-time PCR.

Results & Discussion:

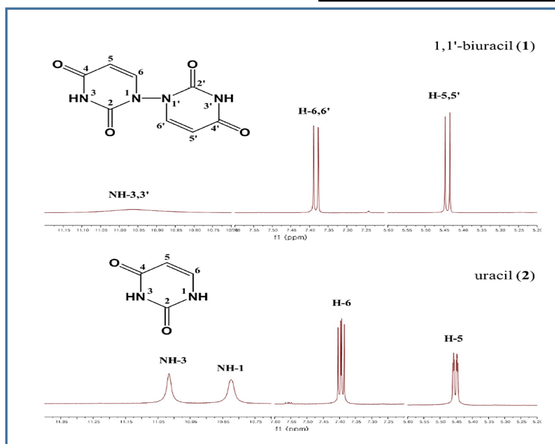

Fig. 1 ^{1}H-NMR spectrum of 1,1′-biuracil (1) and uracil (2) (600 MHz, DMSO-$_{d6}$)

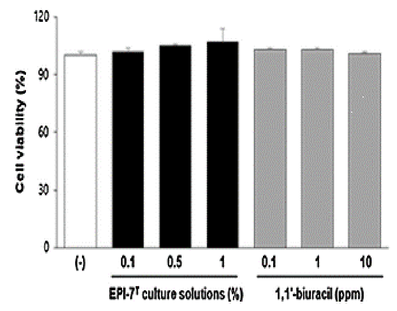

Fig. 2 Cell viability of EPI-7^T culture solutions and 1, 1′-biuracil in Hs68 human fibroblasts

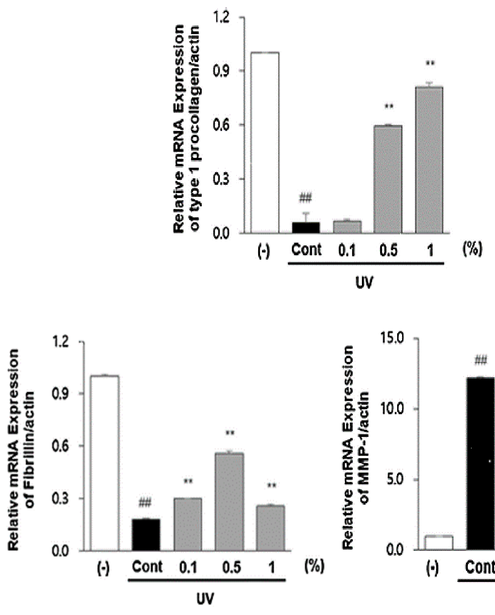

Fig. 3 The effect of EPI-7^T culture solutions on the regulation of anti-aging associated mRNA expressions in UV-irradiated fibroblasts

Fig. 4 Anti-aging functions of 1,1′-biuracil in UV-irradiated fibroblasts

Conclusions:

- In this study, we collected skin-microbiome samples from 20s and 40s age groups of females. We found that the younger skin appeared to have high proportion of *Epidermidibacterium keratini* called EPI-7^T, while the older skin had no EPI-7^T but rather other types of bacteria.
- By repeated column separation, one new pyrimidine compound, 1,1′-biuracil, from EPI-7^T culture solutions grown in R2A medium was yielded.
- EPI-7^T culture solutions increased type I procollagen and fibrillin expressions which were suppressed by UV irradiation. In addition, the expression level of MMP-1 was significantly reduced without cell toxicity.
- Application of 1,1′-biuracil derived from EPI-7^T culture solutions did not regulate type I procollagen or MMP-1 mRNA expression (data not shown), whereas it significantly increased fibrillin expression and reduced that of MMP-3, the fibrillin-degrading proteinase.
- Taken together, these results suggest that 1,1′-biuracil is a key molecule in EPI-7^T culture solutions, exerting protective effects against UV-irradiated skin aging.

Aknowledgments:

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