Anti-Aging Activity of *Lavandula angustifolia* Extract Fermented with *Pediococcus pentosaceus* DK1 Isolated from *Diospyros kaki* Fruit in UVB-Irradiated Human Skin Fibroblasts and Analysis of Principal Components

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The effects of *Lavandula angustifolia* extract fermented with *Pediococcus pentosaceus* DK1 on UVB-mediated MMP-1 expression and collagen decrease in human skin fibroblasts were determined, and the conversion of its components was also analyzed. Fermentation was performed at varying *L. angustifolia* extract and MRS medium concentrations, and optimal fermentation conditions were selected. *L. angustifolia* extracts showed decreased cytotoxicity after fermentation in the fibroblasts. UVB-irradiated fibroblasts treated with fermented *L. angustifolia* extract showed MMP-1 expression 8.2-14.0% lower than that in UVB-irradiated fibroblasts treated with non-fermented extract. This was observed even at fermented extract concentrations lower than those of non-fermented extracts. Fibroblasts treated with fermented *L. angustifolia* extract showed 20% less reduction in collagen production upon UVB irradiation than those treated with non-fermented extracts. UVB-irradiated fibroblasts treated with fermented *L. angustifolia* extracts showed 50% higher inhibition of ROS generation than those treated with non-fermented extract. Luteolin and apigenin glycosides of *L. angustifolia* were converted during fermentation, and identified using RP-HPLC and LC/ESI-MS. Therefore, the effects of *L. angustifolia* extract on MMP-1 expression and collagen decrease in UVB-irradiated human skin fibroblasts were increased through fermentation by *P. pentosaceus*.

**Keywords:** *Pediococcus pentosaceus* DK1, fermentation, UVB, matrix metalloproteinase-1, procollagen

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**Introduction**

The skin forms the external surface of the human body, and serves as a barrier protecting the internal organs from ultraviolet radiation, toxins, and bacteria, etc. There are two dependent layers of the skin, the epidermis and dermis, and these consist of many cells such as keratinocytes, melanocytes and fibroblasts [1]. The mechanical strength of the skin is contributed by the dermis, which is composed of the extracellular matrix (ECM) where fibroblasts synthesize ECM components such as collagen and elastin to sustain the skin’s elasticity [2]. However, the structure and function of the dermis can be changed by harmful external factors such as oxidative stress, UV exposure and air pollution. These conditions accelerate aging of the skin by collapsing its dermal structure [3, 4].

Ultraviolet light is a significant cause of exogenous skin damage. The ultraviolet rays that reach the Earth are classified as UVA (320–400 nm) and UVB (280–320 nm) [5]. When exposed to UV on the Earth’s surface, the amount of UV radiation that reaches the human skin is known to be 25 J/cm\(^2\) under natural sunlight in autumn at
38° N for 4–5 h. It has been reported that this corresponds to ten times the minimum erythema dose in skin [6]. In particular, UVB can penetrate the upper layer of the dermis [7]. UVB exposure increases reactive oxygen species (ROS), such as hydroxyl radicals (•OH), superoxide anion radicals (O2• −), singlet oxygen (1O2), and hydrogen peroxide (H2O2).

To prevent ROS-induced cellular damage, enzymes (superoxide dismutase and catalase) and non-enzymatic antioxidants (L-ascorbic acid and α-tocopherol) are present in skin cells [8, 9]. However, when the balance of this ROS/antioxidant defense system is upset due to excess ROS generation caused by UVB exposure, skin cells become damaged and skin aging is accelerated [10, 11].

ROS induced by UVB exposure increase the expression of matrix metalloproteinase-1 (MMP-1) in fibroblasts, promoting skin photo-aging [12–14]. MMP-1 degrades collagen type 1, which is an ECM component that provides structural support to the skin. This leads to disintegration of the dermis and acceleration of skin aging [15]. Therefore, the development of anti-aging agents to inhibit UVB-induced ROS generation is an essential strategy for suppressing photo-aging [16].

Pediococcus pentosaceus as a lactobacillus is commonly found in fermented foods such as doenjang and kimchi, and is known to produce lactic acid through anaerobic fermentation [17]. Recently, it was reported that P. pentosaceus enhanced the immune activity of Cordyceps militaris extract, enabling it to boost the phagocytic activity of macrophages in mouse [18]. P. pentosaceus increased the anti-aging activity of Gelidium amansil extract on MMP-1 expression and decrease of collagen by UVB irradiation [19]. We isolated and identified a new lactobacillus, P. pentosaceus DK1, from Diospyros kaki fruit. P. pentosaceus DK1 donated to KCTC (KCTC12963BP) could degrade the tannins in Diospyros kaki fruit and increase the total phenol content by 1.5 fold, and the flavonoid content by 1.4 fold. Diospyros kaki fruit fermented by P. pentosaceus DK1 also enhanced the inhibitory activity of elastase for improvement of skin aging. Thus, P. pentosaceus DK1 was also used to improve the anti-aging activity of L. angustifolia.

L. angustifolia, commonly known as lavender, belongs to the Lamiaceae family [20]. Traditionally, L. angustifolia extract has been used as a remedy for neurological and rheumatic diseases, due to its antibacterial and relaxing properties, and is mainly obtained by extracting volatile components from oils [21, 22]. Another method involves extracting L. angustifolia using water or ethanol, which has been reported to produce extracts that show antioxidant properties, whitening effect, and inhibitory effect on sebum production [23]. The major components of L. angustifolia extract are linalool, linalyl acetate, ladanein, apigenin, apigenin-7-O-β-glucoside, luteolin, luteolin-7-O-β-glucoside, and 5,4′-dihydroxy flavonoid-7-O-β-pyrylglycuronate butyl ester [24, 25]. Recently, Ahn et al. [26] reported that the antioxidant, tyrosinase, and elastase inhibitory activities of L. angustifolia extract, as well as the ratios of phenolic compounds such as rosmarinic acid, were significantly increased by natural fermentation. However, their fermentation method was very old, and the microorganisms used were also not identified. There have been no studies so far on the anti-aging activity and composition analysis of L. angustifolia extracts fermented by P. pentosaceus DK1.

In this study, the anti-aging effects of L. angustifolia extract fermented by P. pentosaceus DK1 were evaluated on MMP-1 expression, collagen production, and antioxidant activity, and analyzed on the converted components during fermentation.

**Materials and Methods**

**Reagents and Chemicals**

Human skin fibroblasts (HS68 cells) were purchased from Lonza (Basel, Switzerland). Dulbecco’s modified Eagle’s medium (DMEM), foetal bovine serum (FBS), trypsin, and penicillin-streptomycin were obtained from Capricorn Scientific (Ebsdorfergrund, Germany).

**Preparation of L. angustifolia Extract**

Fermented and non-fermented L. angustifolia extract were obtained from GFC Life Science (Korea). The dried L. angustifolia flowers (2 kg) were homogenized in 70% ethanol (40 L) for 20 days at room temperature. The extract powder (410.93 g) was obtained by filtration and vacuum evaporation drying.

**Fermentation of L. angustifolia Extract**

In order to cultivate the P. pentosaceus DK1 strain, lactobacilli MRS broth (BD 288130) purchased from Difco (USA) was selected as the culture medium for the optimal growth conditions of the strain. MRS medium was developed to favor the growth of lactobacilli in 1960 by De Man, Rogosa and Sharpe and is known to support the growth of lactobacilli including Pediococcus. MRS broth was dissolved in distilled water, and L. angustifolia extract was added (Table 1). P. pentosaceus DK1 was pre-cultured in MRS broth and cultured at 37°C for 24 h. Then, 10% of the strains pre-cultured in the broth containing the L. angustifolia extract were cultured at 37°C for 14 d. The fermented broth was then centrifuged at 9,010 g for 15 min and the supernatant was filtered using a 0.22 μm cellulose filter. Finally, the fermented broth was fractioned with ethyl acetate. The ethyl acetate fraction was evaporated to obtain fermented L. angustifolia extract. All the processes were indicated in Scheme 1.