

【Invention Title】

COMPOSITION FOR TREATING SKIN CELL DAMAGE CAUSED BY FINE
DUST, CONTAINING GREEN TEA-DERIVED LACTIC ACID BACTERIA

5 **【Technical Field】**

Disclosed in the present specification is a composition for treating skin cell damage caused by fine dust. More particularly, disclosed is a composition comprising green tea-derived lactic acid bacteria, which treats skin cell damage by significantly changing biomarkers, etc., which are genes in skin cells, the expression
10 of which is affected by fine dust as compared to the skin cells under normal conditions.

【Background Art】

Recently, interests in fine dust as an environmental issue are increasing and
15 the pollution market is growing. Fine dust can penetrate deeper into the human body because it has a smaller size than Asian dust, etc. and is harmful to the human body because it contains more harmful substances such as heavy metals, nitrates, ammonium salts, sulfates, etc. When the fine dust enters the human body, immune cells in the body remove the dust. During this process, inflammation occurs as a
20 side effect. In addition, the World Health Organization classifies fine dust as a Group 1 carcinogen which is directly related with cancer.

It is expected that the fine dust causes skin troubles and hair loss as it penetrates not only into the respiratory tract but also into the skin or hair follicles.

However, researches on the mechanism by which it negatively affects the skin and hair and on its targets are insufficient, and researches on anti-pollution substances for solving the problem are nonexistent.

Non-patent document 1 reports that the expression amount of claudin 1 (CLDN 1), which is the main component of the tight junction serving as the barrier of the granular layer is decreased by the stimulation of PM_{2.5} fine dust.

Lactic acid bacteria refer to the bacteria that produce lactic acid by decomposing sugars such as glucose, etc. The lactic acid produced from lactic acid fermentation by the lactic acid bacteria can prevent the growth of pathogens and harmful bacteria. For this reason, it is used when preparing foods such as dairy products, kimchis, brewed foods, etc. In addition, the lactic acid bacteria are important bacteria used as a probiotic since they inhabit the intestines of mammals and prevent abnormal fermentation caused by harmful bacteria.

Lactobacillus plantarum is one of lactic acid bacteria and is known to grow mainly when the fermentation of kimchi has proceeded considerably and it tastes sour. It produces both optical isomers (D and L) of lactic acid. *Lactobacillus plantarum* is known for its superior acid tolerance, bile acid resistance and antibacterial activity since it can be widely used for various foods requiring fermentation. However, its use for antipollution such as the effect of strengthening the skin barrier, restoring skin cell damage, etc. is not known.

References of Related Art

Non-patent Documents

(Non-patent document 1) Kim, H.J., et al, "Transcriptome analysis of airborne PM_{2.5}-induced detrimental effects on human keratinocytes", *Toxicology Letters* 273,

26-35, 2017.

【Detailed Description of the Invention】

【Technical Problem】

5 The inventors of the present disclosure have identified that fine dust has a harmful effect on skin and symptoms such as skin cell damage, etc. occur as the expression of genes in skin cells is affected by the effect, and that green tea-derived lactic acid bacteria have an effect of restoring the expression of the genes.

10 In an aspect, the present disclosure is directed to providing a composition for treating skin cell damage caused by fine dust, which comprises green tea-derived lactic acid bacteria, a lysate thereof, a culture thereof or an extract thereof.

【Technical Solution】

15 In an aspect, the present disclosure provides a composition for treating skin cell damage caused by fine dust, which comprises, as an active ingredient, green tea-derived lactic acid bacteria, a lysate thereof, a culture thereof or an extract thereof, and adjusts the expression amount of one or more selected from a group consisting of keratin 1 (KRT 1), claudin 1 (CLDN 1), claudin 4 (CLDN 4) and occludin (OCLN), which are genes in skin cells, the expression amount of which is affected by
20 fine dust, to a normal level.

【Advantageous Effects】

 In an aspect, by using a composition for treating skin cell damage caused by

fine dust provided by the present disclosure, the gene expression amount changed by fine dust can be returned to a normal level such that skin cell damage can be treated.

5 **【Brief Description of Drawings】**

FIG. 1A shows that the mRNA expression amount of the claudin 1 (CLDN 1) gene in skin cells is increased by treatment with a type strain or green tea-derived lactic acid bacteria.

10 FIG. 1B shows that the mRNA expression amount of the claudin 4 (CLDN 4) gene in skin cells is increased by treatment with a type strain or green tea-derived lactic acid bacteria.

FIG. 1C shows that the mRNA expression amount of the occludin (OCLN) gene in skin cells is increased by treatment with a type strain or green tea-derived lactic acid bacteria.

15 FIG. 1D shows that the mRNA expression amount of the keratin 1 (KRT 1) gene in skin cells is increased by treatment with a type strain or green tea-derived lactic acid bacteria.

FIG. 2A shows that the mRNA expression amount of the claudin 1 (CLDN 1) gene is decreased in skin cells stimulated by ERM-CZ120 fine dust and is restored
20 to a normal level by treatment with green tea-derived lactic acid bacteria.

FIG. 2B shows that the mRNA expression amount of the claudin 4 (CLDN 4) gene is decreased in skin cells stimulated by ERM-CZ120 fine dust and is restored to a normal level by treatment with green tea-derived lactic acid bacteria.

FIG. 2C shows that the mRNA expression amount of the occludin (OCLN)

gene is decreased in skin cells stimulated by ERM-CZ120 fine dust and is restored to a normal level by treatment with green tea-derived lactic acid bacteria.

FIG. 2D shows that the mRNA expression amount of the keratin 1 (KRT 1) gene is decreased in skin cells stimulated by ERM-CZ120 fine dust and is restored
5 to a normal level by treatment with green tea-derived lactic acid bacteria.

【Detailed Description of the Preferred Embodiments】

Hereinafter, the present disclosure is described in detail.

In an aspect of the present disclosure, a composition for treating skin cell
10 damage caused by fine dust may comprise green tea-derived lactic acid bacteria, a lysate thereof, a culture thereof or an extract thereof as an active ingredient.

In the present specification, “green tea-derived lactic acid bacteria” may refer to lactic acid bacteria isolated from the leaf of tea plant, the flower of tea plant, the stem of tea plant, the root of tea plant, nearby soil, etc. For example, it may refer to
15 lactic acid bacteria cultured and isolated from green tea, or the leaf of tea plant.

In the present specification, a “strain lysate” may refer to product obtained by lysing a strain itself chemically or with physical force.

In the present specification, a “culture” may refer to a substance including all the substances contained in a medium wherein a strain has been cultured. For
20 example, it may refer to a substance including a metabolite or a secreted product resulting from the culturing of a strain or a lysate thereof, and the strain itself may be included in the culture.

In the present specification, an “extract” may refer to a product obtained by extracting a strain itself, a lysate of the strain, a culture thereof or a mixture thereof

using an extraction solvent.

The green tea-derived lactic acid bacteria according to an exemplary embodiment of the present disclosure refer to lactic acid bacteria isolated from the leaf of tea plant. They have very superior effect of restoring the skin damaged by fine dust, specifically when applied to the tight junction of the skin barrier.

In an aspect, the green tea-derived lactic acid bacteria may be lactic acid bacteria in the genus *Lactobacillus* (*Lactobacillus* sp.). Specifically, the green tea-derived lactic acid bacteria may be *Lactobacillus plantarum*. More specifically, the green tea-derived lactic acid bacteria may be *Lactobacillus plantarum* APSulloc 331266 (KCCM11181P). The green tea-derived lactic acid bacteria may strengthen the tight junction of skin barrier and, in particular, *Lactobacillus plantarum* APSulloc 331266 (KCCM11181P) may have an effect of restoring the skin barrier damaged by fine dust.

In an aspect, the active ingredient of the composition may be an apoptotic body of green tea-derived lactic acid bacteria.

In an aspect of the present disclosure, the composition may contain the green tea-derived lactic acid bacteria, the lysate thereof, the culture thereof or the extract thereof in an amount of 0.000001-30 wt% based on the total weight of the composition. When the content is 0.000001-30 wt%, the green tea-derived lactic acid bacteria exhibit superior effect of treating skin cell damage caused by fine dust.

Specifically, the content may be 0.0000001 wt% or more, 0.0000005 wt% or more, 0.0000007 wt% or more, 0.0000009 wt% or more, 0.000001 wt% or more, 0.000002 wt% or more, 0.000004 wt% or more, 0.000006 wt% or more, 0.000008 wt% or more, 0.00001 wt% or more, 0.00003 wt% or more, 0.00005 wt% or more, 0.00007 wt% or more, 0.00009 wt% or more, 0.0001 wt% or more, 0.0003 wt% or

more, 0.0005 wt% or more, 0.0007 wt% or more, 0.0009 wt% or more, 0.001 wt% or more, 0.01 wt% or more, 0.1 wt% or more, 1 wt% or more, 3 wt% or more, 5 wt% or more, 7 wt% or more, 9 wt% or more, 10 wt% or more, 13 wt% or more, 15 wt% or more, 17 wt% or more, 19 wt% or more, 21 wt% or more, 23 wt% or more, 25 wt% or more, 27 wt% or more, 29 wt% or more, 30 wt% or more or 31 wt% or more, and may be 32 wt% or less, 31 wt% or less, 30 wt% or less, 29 wt% or less, 28 wt% or less, 26 wt% or less, 24 wt% or less, 22 wt% or less, 20 wt% or less, 18 wt% or less, 16 wt% or less, 14 wt% or less, 12 wt% or less, 10 wt% or less, 9 wt% or less, 8 wt% or less, 6 wt% or less, 4 wt% or less, 2 wt% or less, 1 wt% or less, 0.1 wt% or less, 0.09 wt% or less, 0.04 wt% or less, 0.01 wt% or less, 0.006 wt% or less, 0.001 wt% or less, 0.0009 wt% or less, 0.0007 wt% or less, 0.00005 wt% or less, 0.00003 wt% or less, 0.00001 wt% or less, 0.000009 wt% or less, 0.000007 wt% or less, 0.000005 wt% or less, 0.000003 wt% or less, 0.000001 wt% or less, 0.0000009 wt% or less, 0.0000007 wt% or less, 0.0000005 wt% or less, 0.0000003 wt% or less, 0.0000001 wt% or less or 0.00000009 wt% or less, although not being limited thereto.

In an aspect, the active ingredient of the composition according to the present disclosure may act on the tight junction of the skin barrier.

Tight junction is a type of cell junction and is composed of occludin, claudin, tricellulin, junctional adhesion molecule (JAM), zona occluden (ZO), etc. The tight junction can be divided into a transmembrane part and an intracellular part. Occludin and claudin are constitutive proteins of the tight junction included in the cell membrane. Occludin is the first transmembrane protein found in the tight junction and consists of four transmembrane domains. Occludin is known not only as a component of the tight junction but also as regulating the function of the tight junction.

Claudin, which is a constitutive protein of the tight junction together with occludin, also has four transmembrane domains. The tight junction fills the gap between adjacent cells and regulates the transport of small materials. It is also involved in the regulation of penetration of materials between cells and maintenance of cell polarity. Recently, it was reported that the tight junction plays an important role in the skin barrier function. The tight junction is a type of adhesion between cells. It is a cell junction occurring mainly in epithelial cells and endothelial cells and forms a complicated network having a string-shaped structure. Due to this structure, the tight junction largely has two functions. The first is a barrier function which prevents leakage of materials between epithelial cells or endothelial cells. This is considered important mainly in blood vessels or the digestive tract. The second is an enclosure function of surrounding the cell membrane lipid in the shape of a string and compartmenting the cell membrane. Accordingly, the active ingredient of the composition according to the present disclosure can treat skin cell damage caused by fine dust by acting on the tight junction of the skin barrier, thereby enhancing the barrier function and enclosure function of skin cells.

Another aspect of the present disclosure includes a use of the composition for treating skin cell damage caused by fine dust.

In another aspect, the present disclosure provides a method for treating skin cell damage of a subject caused by fine dust, comprising administering a composition comprising a green tea-derived lactic acid bacteria, a lysate thereof, a culture thereof or an extract thereof to a subject in need thereof.

In another aspect, the present disclosure provides a use of green tea-derived lactic acid bacteria, a lysate thereof, a culture thereof or an extract thereof in the manufacture of a composition for treating skin cell damage caused by fine dust.

In another aspect, the present disclosure provides a green tea-derived lactic acid bacteria, a lysate thereof, a culture thereof or an extract thereof for treating skin cell damage caused by fine dust.

In the present specification, “fine dust” refers to a very small material that is invisible to the naked eye. It is a particulate matter that floats or flutters in the air for a long time. Specifically, in the present disclosure, the fine dust may have a particle diameter of 10 μm or smaller (PM_{10}), specifically a particle diameter of 2.5 μm or smaller ($\text{PM}_{2.5}$). In particular, a particulate matter having a particle diameter of 2.5 μm or smaller ($\text{PM}_{2.5}$) is called “ultrafine dust”. In the present specification, the “fine dust” is intended to include the “ultrafine dust”.

In an aspect, the fine dust in the present disclosure may include one or more of arsenic, cadmium, lead and nickel. The heavy metal components such as arsenic, cadmium, lead, nickel, etc. included in the fine dust can induce skin aging by attacking skin cells and causing skin allergy, pigmentation, inflammation, etc. In an exemplary embodiment, the fine dust includes arsenic, cadmium, lead and nickel.

In the present specification, the term “treatment” refers to effective protection of skin cells from irritation and inhibition, prevention and restoration (recovery) of the change of the expression amount of a specific gene due to the irritation. Here, “restoration” includes, in addition to change of the expression amount close to the reference value of the gene expression amount in the absence of irritation, improvement of the expression amount above the reference value.

In an aspect, the present disclosure provides a composition for inhibiting skin cell damage caused by fine dust, which adjusts the expression amount of a specific gene in skin cells, the expression amount of which is affected by fine dust, to a normal level.

Specifically, in the present disclosure, the gene in skin cells, the expression amount of which is affected by fine dust, may include one or more selected from a group consisting of keratin 1 (KRT 1), claudin 1 (CLDN 1), claudin 4 (CLDN 4) and occludin (OCLN). Since keratin 1 (KRT 1), claudin 1 (CLDN 1), claudin 4 (CLDN 4) and occludin (OCLN) are the genes, the expression amount of which is decreased by fine dust, skin cell damage can be inhibited by adjusting the expression amount of these genes to a normal level.

In an aspect, the composition according to the present disclosure may have an effect of strengthening the skin barrier or restoring the skin barrier.

The genes used in the present disclosure, the expression amount of which is changed by fine dust, are described in Table 1. Table 1 shows the genes, the expression amount of which is decreased by fine dust. In the table, the gene symbol means the official gene symbol, and the gene title means the name of each gene. These are described in the non-patent document 1.

[Table 1]

Decreased genes	
Gene symbol	Gene title
KRT 1	Keratin 1
CLDN 1	Claudin 1
CLDN 4	Claudin 4
OCLN	Occludin

The expression amount of these genes or proteins may be analyzed using various analysis methods known in the art, such as microarray, PCR, NGS (next-generation sequencing), western blot, northern blot, ELISA, radioimmunoassay,

radioimmunodiffusion, immunohistochemical staining, immunoprecipitation assay, etc.

Fine dust causes skin cell damage, which leads to inflammation and thus further damage to skin cells. The skin condition can be improved by taking care of this vicious cycle of skin cell damage with green tea-derived lactic acid bacteria.

5 In an aspect of the present disclosure, the composition may be a cosmetic composition, a pharmaceutical composition or a health functional food composition.

For example, the cosmetic composition may be a cosmetic such as a cream, a lotion, a toner, a cleanser, a face cleanser, a soap, a cosmetic solution, etc.

10 In an aspect, the cosmetic containing the composition of the present disclosure comprising the green tea-derived lactic acid bacteria may be in the form of a solution, an emulsion, a viscous mixture, etc.

That is to say, in an aspect, the formulation of the cosmetic of the present disclosure is not particularly limited. For example, the formulation may be an emulsion, a cream, a toner, an essence, a pack, a gel, a powder, a makeup base, a foundation, a lotion, an ointment, a patch, a cosmetic solution, a cleansing foam, a cleansing cream, a cleansing water, a body lotion, a body cream, a body oil, a body essence, a shampoo, a rinse, a body cleanser, a soap, a hair dye, a spray, etc.

20 Ingredients other than the green tea-derived lactic acid bacteria may be selected and added to the cosmetic composition of each formulation without difficulty by those skilled in the art in consideration of the formulation or purpose of use.

In addition, in an aspect, the cosmetic of the present disclosure may contain one selected from a group consisting of a water-soluble vitamin, an oil-soluble vitamin, a polypeptide, a polysaccharide, a sphingolipid and a seaweed extract.

25 In addition, in an aspect, the cosmetic of the present disclosure may contain ingredients that are generally used in cosmetics in addition to the essential

ingredient, if necessary.

Examples of the additional ingredients include oils and fats, moisturizers, emollients, surfactants, organic and inorganic pigments, organic powders, UV absorbers, preservatives, sterilizers, antioxidants, plant extracts, pH adjusters, alcohols, colorants, fragrance, blood circulation stimulants, skin coolers, antiperspirants, purified water, etc.

However, the ingredients that may be contained in the cosmetic are not limited thereto. Also, the amount of any of the ingredients may be determined within a range not negatively affecting the purpose and effect of the present disclosure.

In an aspect, the pharmaceutical composition containing green tea-derived lactic acid bacteria of the present disclosure may further comprise a suitable carrier, excipient and diluent commonly used for preparation of pharmaceutical compositions.

The pharmaceutical composition comprising the green tea-derived lactic acid bacteria may be formulated into any form suitable for pharmaceutical preparations, including oral formulations such as a tablet, a capsule, a powder, a syrup, etc. and agents for external application to the skin such as an ointment, a gel, a cream, a patch, a spray, etc. according to common methods.

In general, it is to be understood that the actual dosage of the active ingredient administered by the pharmaceutical composition should be determined in light of various relevant factors such as the severity of the symptom, the selected administration route, the age, gender, body weight and health condition of a subject, etc. In general, the dosage of the active ingredient may be 0.0001-3000 mg/kg/day, for example, 10-500 mg/kg/day.

In the health functional food composition according to an aspect of the

present disclosure, the health food may refer to a food prepared from nutrients which are likely to be deficient in normal diets or raw materials or ingredients (functional raw materials) with functions useful for the human body, and which maintain and improve health by maintaining the normal function of the human body or activating physiological functions, although not being limited thereto. The health food may be prepared and processed into the form of a tablet, a capsule, a powder, a granule, a liquid, a pill, etc. However, the formulation is not limited thereto, and it may be prepared and processed into any form under the law.

Specifically, a health beverage composition is not particularly limited in ingredients other than the above-described compound contained in the predetermined ratio as an essential ingredient. It may contain various flavoring agents or natural carbohydrates as additional ingredients as in common beverages. Examples of the natural carbohydrates are conventional sugars such as a monosaccharide, a polysaccharide, a cyclodextrin, etc. and sugar alcohols such as xylitol, sorbitol, erythritol, etc. Also, natural flavoring agents (thaumatin, stevia extract (e.g., rebaudioside A, glycyrrhizin, etc.)) and synthetic flavoring agents (e.g., saccharin, aspartame, etc.) may be used as the flavoring agent.

In general, it is to be understood that the actual dosage of the active ingredient administered by the health functional food composition should be determined in light of various relevant factors such as the severity of the symptom, the selected administration route, the age, gender, body weight and health condition of a subject, etc. In general, the dosage of the active ingredient may be 0.0001-1000 mg/kg/day, for example, 0.02-6 mg/kg/day.

Hereinafter, the constitution and effect of the present disclosure will be

described in more detail with reference to examples. However, the following examples are provided for illustrative purposes only to facilitate understanding of the present disclosure, and the scope of the present disclosure is not limited thereto.

5 [Preparation Example1] *Lactobacillus plantarum* APsulloc 331266

1. Culturing and isolation of *Lactobacillus plantarum* APsulloc 331266

200 g of the leaf of tea plant was washed twice with single distilled water to remove foreign materials. After removing water from the washed leaf of tea plant, it was mixed with salt corresponding to 8% of the weight of the leaf of tea plant and
10 kept at room temperature for 3 hours. The salted leaf of tea plant was mixed with 1000 mL of a 1% fructooligosaccharide solution and cultured in an incubator at 32 °C for 3 days. 3 days later, it was investigated whether the pH of the culture was decreased below 5. If the pH was decreased below 5, the culture was taken and cultured in a Difco Lactobacilli MRS Agar® medium. The culturing was conducted
15 at 32 °C in a chamber of anaerobic condition for 2 days and a white colony was taken.

Lactobacillus plantarum APsulloc 331266 was isolated from the leaf of tea plant according to the method described above. The *Lactobacillus plantarum* APsulloc 331266 was deposited on March 28, 2011 in the Korean Culture Center of
20 Microorganisms under the accession number KCCM11181P.

2. Identification of *Lactobacillus plantarum* APsulloc 331266

(1) Culturing of strain

The APsulloc 331266 isolated in 1 was streaked on a MRS agar plate and
25 then cultured at 37 °C for 2 days. The obtained single colony was inoculated to 10

mL of a MRS broth and an APsulloc 331266 culture was prepared by culturing the same overnight at 37 °C.

(2) Analysis of sugar fermentation pattern of APsulloc 331266

The APsulloc 331266 strain culture prepared in (1) was inoculated to 10 mL of a MRS broth to 0.5% and cultured overnight at 37 °C. The culture was centrifuged at 8,000 rpm for 5 minutes. After removing the supernatant, only the cells were collected and suspended by adding 2 mL of a 0.85% saline buffer. Then, analysis was conducted using an API 50CHL kit (Biomerieux) according to the manufacturer's protocol. Details are as follows.

First, while slowly adding the suspension of the strain to 5 mL of an API suspension medium, the amount of the suspension when the turbidity reached about McFarland standard 2 (Biomerieux) was measured. After adding 10 mL of an API 50CHL medium corresponding to two times of the amount of the suspension measured, the mixture was mixed by shaking. After adding the mixture to cupules containing different substrates and dropping one drop of mineral oil, sugar fermentation pattern was analyzed after conducting culturing at 37 °C for 2 days.

The result of analyzing the sugar fermentation pattern of APsulloc 331266 using the *Lactobacillus plantarum* strain (KCTC3108) as a type strain and the result of identifying the APsulloc 331266 are shown in the following table.

[Table 2]

Substrate	KCTC3108		APsulloc 331266		Substrate	KCTC3108		APsulloc 331266	
	24h	48h	24h	48h		24h	48h	24h	48h

Control	-	-	-	-	Esculin	+	+	+	+
Glycerol	-	-	-	-	Salicin	+	+	+	+
Erythritol	-	-	-	-	Cellobiose	?	+	+	+
D-Arabinose	-	-	-	-	Maltose	+	+	+	+
L-Arabinose	+	+	+	+	Lactose	+	+	+	+
Ribose	+	+	+	+	Melibiose	+	+	+	+
D-Xylose	-	-	-	-	D-Saccharose (sucrose)	+	+	+	+
L-Xylose	-	-	-	-	Trehalose	+	+	+	+
Adonitol	-	-	-	-	Inulin	-	-	-	-
β -Methyl-D-xylose	-	-	-	-	Melezitose	+	+	+	+
Galactose	+	+	+	+	Raffinose	-	-	+	+
Glucose	+	+	+	+	Amidone (starch)	-	-	-	-
Fructose	+	+	+	+	Glycogen	-	-	-	-
Mannose	+	+	+	+	Xylitol	-	-	-	-
Sorbose	-	-	-	-	Gentiobiose	-	-	+	+
Rhamnose	-	-	-	-	D-Turanose	+	+	+	+
Dulcitol	-	-	-	-	D-Lyxose	-	-	-	-
Inositol	-	-	-	-	D-Tagatose	-	-	-	-
Mannitol	+	+	+	+	D-Fucose	-	-	-	-
Sorbitol	+	+	+	+	L-Fucose	-	-	-	-
α -Ethyl-D-	?	+	-	-	D-Arabitol	?	-	-	-

mannoside									
α -Ethyl-D-glucoside	-	-	-	-	L-Arabitol	-	-	-	-
N-Acetyl-glucosamine	+	+	+	+	Gluconic acid	?	+	+	+
Amygdalin	+	+	+	+	2-Ketogluconate	-	-	-	-
Arbutin	+	+	+	+	5-Ketogluconate	-	-	-	-

+: substrate degraded, -: substrate not degraded, ?: undeterminable

[Table 3]

Strain	Scientific name	% index	T index
KCTC	<i>Lactobacillus plantarum</i>	99.9	0.8
3108	<i>Lactobacillus pentosus</i>	0.1	0.29
APsulloc	<i>Lactobacillus plantarum</i>	99.4	0.99
331266	<i>Lactobacillus pentosus</i>	0.4	0.71

As can be seen from above, APsulloc 331266 showed 99% or more identity (% index) to *Lactobacillus plantarum*, suggesting that it is *Lactobacillus plantarum*.

- 5 In addition, APsulloc 331266 showed difference in use of α -methyl-mannoside (mannoside) and raffinose from the type strain (KCTC3108), suggesting that they are different strains.

3. Preparation of apoptotic body of *Lactobacillus plantarum* APsulloc 331266

- 10 The isolated and identified *Lactobacillus plantarum* APsulloc 33126 was

cultured in a MRS broth at 37 °C for 24 hours. The lactic acid bacteria was inoculated into a cryotube with 10 wt% skim milk. When the concentration of the bacteria in the liquid medium reached 5×10^8 to 1×10^9 CFU/mL, the cells were harvested after centrifuging at 4 °C and 4000 rpm and then washed with PBS (phosphate-buffered saline). Then, the washed cells were resuspended in PBS at a concentration of 5×10^8 to 1×10^9 CFU/mL and then the apoptotic body of *Lactobacillus plantarum* was prepared by heating at 100 °C for 10 minutes.

[Experimental Example 1] Measurement of gene expression amount through real-time qPCR

1. Culturing of keratinocytes

Human normal epidermal keratinocytes were purchased from Lonza Inc. (Walkersville, Maryland, USA), subcultured and then cultured in a CO₂ incubator under the condition of 37 °C and 5% CO₂. The cell culturing was performed using 500 mL of a KBM-2 (KBMTM-2, CC-3103) medium containing BPE (bovine pituitary extract), human epidermal growth factor (hEGF), insulin, hydrocortisone, gentamicin sulfate, epinephrine and transferrin.

During the culturing, the maximum confluency was maintained at about 90%. Then, subculturing was performed.

2. Treatment of keratinocytes with fine dust and *Lactobacillus plantarum*

As fine dust, ERM-CZ120 fine dust commercially available from Sigma-Aldrich was used. It included PM₁₀ fine dust together with arsenic, cadmium, lead and nickel.

A 6-well plate was used. The cells cultured to 3×10^5 cells/well under the

condition of '1. Culturing of keratinocytes' were treated with nothing (Control 1), with 50 ppm fine dust (Control 2), with 1 µg/mL *Lactobacillus plantarum* of Preparation Example1 (Example 1), with 50 ppm fine dust and 1 µg/mL *Lactobacillus plantarum* of Preparation Example1 (Example 2), with 1 µg/mL *Lactobacillus plantarum* type strain (Comparative Example 1), or with 50 ppm fine dust and 1 µg/mL *Lactobacillus plantarum* type strain (Comparative Example 2), as a total of 6 groups.

As the *Lactobacillus plantarum* type strain, the strain deposited with the accession number KCTC3108 was obtained from the Korean Collection for Type Cultures.

[Table 4]

Experimental groups	ERM-CZ120 fine dust	APsulloc 331266	Type strain
Control 1 (not treated with fine dust)	-	-	-
Control 2 (treated with fine dust)	50 ppm	-	-
Example 1 (treated with APsulloc 331266 of Preparation Example1)	-	1 µg/mL	-
Example 2 (treated with fine dust and APsulloc 331266 of Preparation Example1)	50 ppm	1 µg/mL	-
Comparative Example 1	-	-	1 µg/mL

(treated with type strain)			
Comparative Example 2 (treated with fine dust and type strain)	50 ppm	-	1 µg/mL

3. Real-time qPCR

3-1. RNA extraction

After removing the cultures from Control 1, Control 2, Example 1 and Comparative Example 1 obtained in '2. Treatment of keratinocytes with fine dust and *Lactobacillus plantarum*', the cells were washed with 2 mL of phosphate-buffered saline (PBS) and then RNA was isolated from the cells using a Trizol reagent (Invitrogen, Carlsbad, CA, USA).

3-2. Synthesis of cDNA

After purifying the isolated RNA using a Qiagen RNeasy kit (Qiagen, Valencia, CA), the quality of the RNA was determined using the Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). Then, cDNA was synthesized from the RNA using the Superscript Reverse Transcriptase (RT) kit (Invitrogen, Carlsbad, CA).

3-3. qPCR

The cDNA was quantitatively analyzed by real-time reverse transcription polymerase chain reaction (Q-RT-PCR) using primers. The change of gene expression pattern in the cells was evaluated by real-time PCR using a TaqMan gene

expression assay kit (Applied Biosystems, Foster City, CA). The results are shown in FIGS. 1A-2C. Both the Q-RT-PCR and the real-time PCR were performed according to the standard protocols distributed by Life Technologies. Specifically, 40 cycles of 95 °C for 20 seconds, 95 °C for 3 seconds and 60 °C for 30 seconds were used.

4-1. Evaluation of skin barrier enhancing effect of *Lactobacillus plantarum*

As seen from FIGS. 1A-1D, both the type strain and the green tea-derived lactic acid bacteria of the present disclosure were confirmed to have skin barrier enhancing effect when treated alone.

Specifically, it was confirmed that Example 1 and Comparative Example 1 showed increased mRNA expression amount of claudin 1, claudin 4, occludin and keratin 1 as compared to Control 1.

4-2. Evaluation of skin cell damage restoring of *Lactobacillus plantarum*

As seen from FIGS. 2A-2D, the green tea-derived lactic acid bacteria of the present disclosure were confirmed to have restoring skin cell damage caused by fine dust when treated alone.

Specifically, it was confirmed that Example 2 and Comparative Example 2 showed increased mRNA expression amount of claudin 1, claudin 4, occludin and keratin 1 as compared to Control 2. When treated with the fine dust, the mRNA expression amount of keratin 1 was decreased to 0.41, the mRNA expression amount of claudin 1 was decreased to 0.57, the mRNA expression amount of claudin 4 was decreased to 0.50, and the mRNA expression amount of occludin was decreased to 0.42. When treated with the *Lactobacillus plantarum* of Preparation

Example 1, the mRNA expression amount of keratin 1 was restored to 1.01, the mRNA expression amount of claudin 1 was restored to 0.73, the mRNA expression amount of claudin 4 was restored to 1.18, and the mRNA expression amount of occludin was restored to 0.79.

5 In contrast, when treated with the type strain, the change in the mRNA expression was insignificant, with the mRNA expression amount of keratin 1 being 0.51, the mRNA expression amount of claudin 1 being 0.53, the mRNA expression amount of claudin 4 being 0.60, and the mRNA expression amount of occludin being 0.51. Accordingly, it was confirmed that the type strain had no effect of restoring the
10 decreased mRNA expression amount of the markers.

Hereinafter, formulation examples of the composition according to the present disclosure will be described. However, the cosmetic composition, pharmaceutical composition and health functional food composition may be
15 formulated into various other forms. These examples are for illustrative purposes only and are not intended to limit the scope of the present disclosure.

[Formulation Example 1] Tablet

100 mg of the *Lactobacillus plantarum* lactic acid bacteria according to the
20 present disclosure, 400 mg of lactose, 400 mg of corn starch and 2 mg of magnesium stearate were mixed and subjected to a tableting process according to a conventional method for preparing tablets to prepare a tablet.

[Table 5]

Ingredients	Contents (mg)
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<i>Lactobacillus plantarum</i> lactic acid bacteria	100
Lactose	400
Corn starch	400
Magnesium stearate	2

[Formulation Example 2] Capsule

100 mg of the *Lactobacillus plantarum* lactic acid bacteria according the present disclosure, 400 mg of lactose, 400 mg of corn starch and 2 mg of
5 magnesium stearate were mixed and filled in a gelatin capsule according to a conventional method for preparing capsules to prepare a capsule.

[Table 6]

Ingredients	Contents (mg)
<i>Lactobacillus plantarum</i> lactic acid bacteria	100
Lactose	400
Corn starch	400
Magnesium stearate	2

[Formulation Example 3] Granule

10 50 mg of the *Lactobacillus plantarum* lactic acid bacteria according to the present disclosure, 250 mg of anhydrous crystalline glucose and 550 mg of starch were mixed and formulated into granules using a fluidized-bed granulator. The granules were then filled in a pouch.

[Table 7]

Ingredients	Contents (mg)
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<i>Lactobacillus plantarum</i> lactic acid bacteria	50
Anhydrous crystalline glucose	250
Starch	550

[Formulation Example 4] Soap

[Table 8]

Ingredients	Contents (%)
<i>Lactobacillus plantarum</i> lactic acid bacteria	5.00
Oil and fat	q.s.
Sodium hydroxide	q.s.
Sodium chloride	q.s.
Fragrance	q.s.
Purified water	Balance

5 [Formulation Example 5] Lotion

[Table 9]

Ingredients	Contents (%)
<i>Lactobacillus plantarum</i> lactic acid bacteria	5.00
Magnesium L-ascorbic acid-2-phosphate	1.00
Water-soluble collagen (1% aqueous solution)	1.00
Sodium citrate	0.10
Citric acid	0.05
Licorice extract	0.20
1,3-Butylene glycol	3.00

Purified water	Balance
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[Formulation Example 6] Cream

[Table 10]

Ingredients	Contents (%)
<i>Lactobacillus plantarum</i> lactic acid bacteria	3.00
Polyethylene glycol monostearate	2.00
Self-emulsifying glycerin monostearate	5.00
Cetyl alcohol	4.00
Squalene	6.00
<i>Glyceryl tri(2-ethylhexanoate)</i>	6.00
Sphingoglycolipid	1.00
1,3-Butylene glycol	7.00
Purified water	Balance

5 [Formulation Example 7] Ointment

[Table 11]

Ingredients	Contents (%)
<i>Lactobacillus plantarum</i> lactic acid bacteria	5.00
Polyvinyl alcohol	13.00
Magnesium L-ascorbic acid-2-phosphate	1.00
Lauroyl hydroxyproline	1.00
Water-soluble collagen (1% aqueous solution)	2.00
1,3-Butylene glycol	3.00

Ethanol	5.00
Purified water	Balance

[Formulation Example 8] Cosmetic solution

[Table 12]

Ingredients	Contents (%)
<i>Lactobacillus plantarum</i> lactic acid bacteria	3.00
Hydroxyethylene cellulose (2% aqueous solution)	12.00
Xanthan gum (2% aqueous solution)	2.00
1,3-Butylene glycol	6.00
Concentrated glycerin	4.00
Sodium hyaluronate (1% aqueous solution)	2.00
Purified water	Balance

5 [Formulation Example 9] Health food

[Table 13]

Ingredients	Contents
<i>Lactobacillus plantarum</i> lactic acid bacteria	2 mg
Vitamin A acetate	70 µg
Vitamin E	1.0 mg
Vitamin B ₁	0.13 mg
Vitamin B ₂	0.15 mg
Vitamin B ₆	0.5 mg

Vitamin B ₁₂	0.2 µg
Vitamin C	10 mg
Biotin	10 µg
Nicotinamide	1.7 mg
Folic acid	50 µg
Calcium pantothenate	0.5 mg
Ferrous sulfate	1.75 mg
Zinc oxide	0.82 mg
Magnesium carbonate	25.3 mg
Potassium phosphate monobasic	15 mg
Calcium phosphate dibasic	55 mg
Potassium citrate	90mg
Calcium carbonate	100 mg
Magnesium chloride	24.8 mg

[Formulation Example 10] Health beverage

[Table 14]

Ingredients	Contents
<i>Lactobacillus plantarum</i> lactic acid bacteria	50 mg
Citric acid	1000 mg
Oligosaccharide	100 g
Taurine	1 g

Purified water	Balance
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【Accession Number】

Depository authority: Korean Culture Center of Microorganisms

5 Accession number: KCCM11181P

Date of deposit: 20110328

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To, AMOREPACIFIC CORPORATION
181, 2 Ga, Hangang-ro,
Yongsan-Gu,
Seoul 140-777
Republic of Korea

RECEIPT IN THE CASE OF AN ORIGINAL
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : <i>Lactobacillus plantarum</i> APSulloc 331266	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCM11181P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on March. 28. 2011. (date of the original deposit) ¹	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : Korean Culture Center of Microorganisms Address : 361-221, Yurim B/D Hongje-1-dong, Seodaemun-gu SEOUL 120-091 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: March. 28. 2011. 

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired; where a deposit made outside the Budapest Treaty after the acquisition of the status of international depositary authority is converted into a deposit under the Budapest Treaty, such date is the date on which the microorganism was received by the international depositary authority.

[CLAIMS]

【Claim 1】

A cosmetic composition for treating skin cell damage caused by fine dust, comprising green tea-derived lactic acid bacteria, a lysate thereof, a culture thereof or an extract thereof as an active ingredient,

wherein the green tea-derived lactic acid bacteria are *Lactobacillus plantarum* APsulloc 331266 (KCCM11181P).

【Claim 2】

A health functional food cosmetic composition for treating skin cell damage caused by fine dust, comprising green tea-derived lactic acid bacteria, a lysate thereof, a culture thereof or an extract thereof as an active ingredient,

wherein the green tea-derived lactic acid bacteria are *Lactobacillus plantarum* APsulloc 331266 (KCCM11181P).

【Claim 3】

The composition according to claim 1 or 2, wherein the active ingredient is an apoptotic body of green tea-derived lactic acid bacteria.

【Claim 4】

The composition according to claim 1 or 2, wherein the green tea-derived lactic acid bacteria, the lysate thereof, the culture thereof or the extract thereof is comprised in an amount of 0.000001-30 wt% based on the total weight of the composition.

【Claim 5】

The composition according to claim 1 or 2, wherein the active ingredient of the composition acts on the tight junction of the skin barrier.

【Claim 6】

The composition according to claim 1 or 2, wherein the composition restores the expression of one or more of keratin 1 (KRT 1), claudin 1 (CLDN 1), claudin 4 (CLDN 4) and occludin (OCLN).

【Claim 7】

The composition according to claim 1 or 2, wherein the fine dust comprises one or more of arsenic, cadmium, lead and nickel.

【Claim 8】

The composition according to claim 1 or 2, wherein the fine dust has a particle size of PM 10 or less.

【Claim 9】

The composition according to claim 1 or 2, wherein the active ingredient of the composition is to be administered at a dosage of 10-500 mg/kg/day.