(12) STANDARD PATENT (11) Application No. **AU 2021269282 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Novel lactic acid bacteria and use thereof

(51) International Patent Classification(s)
C12N 1/20 (2006.01) ***A61K 35/747*** (2015.01)
A23L 33/135 (2016.01) *C12R 1/01* (2006.01)
A61K 35/745 (2015.01) *C12R 1/25* (2006.01)

(21) Application No: **2021269282** (22) Date of Filing: **2021.11.15**

(43) Publication Date: **2021.12.09**
(43) Publication Journal Date: **2021.12.09**
(44) Accepted Journal Date: **2023.08.17**

(62) Divisional of:
2018358456

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(56) Related Art
KIM D-E. Lactobacillus plantarum NK3 and Bifidobacterium longum NK49 ameliorated Gardnerella vaginalis-induced vaginosis in mice. 2017 FALL INTERNATIONAL CONVENTION OF THE PHARMACEUTICAL SOCIETY OF KOREA . POSTER P 5-13, v. 22(10):1022-1031

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Abstract

The present invention relates to *Lactobacillus plantarum* NK3 and *Bifidobacterium longum* NK49, which are novel lactic acid bacteria and, more particularly, to a composition comprising novel lactic acid bacteria useful for prevention and treatment of inflammatory diseases.

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Specification

Title

NOVEL LACTIC ACID BACTERIA AND USE THEREOF

Related Application

This application is a divisional application of Australian application no. 2018358456, the entire disclosure of which is incorporated herein by reference.

Technical Field

The present invention relates to Lactobacillus plantarum and Bifidobacterium longum, which are novel lactic acid bacteria, and more particularly to a composition containing the novel lactic acid bacteria useful in preventing and treating inflammatory diseases.

Background

Inflammation is a kind of defense reaction which occurs to biological tissues or organs against any external stimuli. In normal cases, the inflammatory reaction removes a material infected from the outside and regenerates a damaged tissue to restore a function thereof. However, if the inflammatory reaction is continuously caused by the secretion of inflammation-inducing cytokines, various inflammatory diseases occur depending on a site where the inflammatory reaction occurs.

On the other hand, vaginitis, which is one of female inflammatory diseases, is an intravaginal infectious disease caused by the growth of various anaerobic bacteria instead of bacteria normally present in the vagina. Recently, the incidence of vaginitis has increased due to women's greater use of feminine products and growing stress. Vaginitis is mostly bacterial vaginosis and fungal vaginitis. Bacterial vaginosis is typically caused by Gardnerella vaginalis or Atopobium vaginae, which is an anaerobic strain. Fungal vaginitis is mainly caused by Candida albicans (Sexually Transmitted Diseases, November 2006, vol 33, No. 11, 663-665).

Meanwhile, in case of bacterial vaginosis, symptoms may be ameliorated by using antibiotics.

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In case of fungal vaginitis, symptoms may be ameliorated by using an azole-based antifungal agent. However, these therapeutic agents may cause a phenomenon of another microbial substitution and thus have a problem in that it is difficult to expect a fundamental therapeutic effect. Thus, there is a need to develop a therapeutic agent capable of suppressing the inflammatory reaction so as to restore normal lactic acid bacteria while removing the vaginitis-causing bacteria from the vagina. However, research has not been sufficiently conducted.

Against these backdrops, while studying agents for preventing and treating inflammatory diseases, particularly vaginitis, the present inventors have identified that novel lactic acid bacteria isolated from kimchi and human feces may be valuably used in preventing and treating inflammatory diseases, particularly vaginitis not only by inhibiting inflammatory reactions, but also by showing a therapeutic effect on vaginitis while inhibiting the growth of vaginitis-causing bacteria, and thus have completed the present invention.

Reference to any prior art in the specification is not an acknowledgement or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be combined with any other piece of prior art by a skilled person in the art.

Detailed Description of the Invention

In any aspect, the invention provides novel lactic acid bacteria of Lactobacillus plantarum and Bifidobacterium longum.

In another aspect, the invention provides a composition for preventing or treating inflammatory diseases, containing novel lactic acid bacteria.

Still in another aspect, the invention provides a health functional food for preventing or ameliorating inflammatory diseases, containing novel lactic acid bacteria.

In one aspect the present invention provides Lactobacillus plantarum NK3 (depository institution: the Korean Culture Center of Microorganisms (KCCM), date of deposit: Aug. 04, 2017, and

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accession number: KCCM12089P).

The Lactobacillus plantarum NK3 of the present invention has a feature of being a novel lactic acid bacterium of Lactobacillus plantarum, which is isolated and identified from kimchi, which is a traditional fermented food.

A 16S rDNA sequence for identification and classification of Lactobacillus plantarum NK3 of the present invention is the same as SEQ ID NO: 1 attached to the present specification. Thus, Lactobacillus plantarum NK3 of the present invention may include the 16S rDNA of SEQ ID NO: 1.

As a result of analyzing said 16S rDNA sequence of SEQ ID NO: 1, this sequence was 99% homologous to that of generally known Lactobacillus plantarum strains, thus showing the highest molecular phylogenetic relationship with Lactobacillus plantarum. Thus, said lactic acid bacterium was identified as Lactobacillus plantarum, then named as Lactobacillus plantarum NK3, and then deposited to the KCCM on Aug. 04, 2017 (accession number: KCCM12089P).

The Lactobacillus plantarum NK3 of the present invention is a gram-positive bacterium and a cell form thereof is bacillus. More particularly, the physiological characteristics of Lactobacillus plantarum NK3 may be analyzed according to a conventional method in the art, and the results thereof are as shown in a following table 2. Particularly, Lactobacillus plantarum NK3 may use the following as a carbon source: L-arabinose, D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, mannitol, sorbitol, α-methyl-D-mannoside, N-acetyl-glucosamine, amygdaline, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, gentiobiose, D-turanose and gluconate.

In another aspect, the present invention provides Bifidobacterium longum NK49 (depository institution: the Korean Culture Center of Microorganisms (KCCM), date of deposit: Aug. 04, 2017, and accession number: KCCM12088P).

The Bifidobacterium longum NK49 of the present invention has a feature of being a novel lactic

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acid bacterium of Bifidobacterium longum, which is isolated and identified from human feces.

A 16S rDNA sequence for identification and classification of Bifidobacterium longum NK49 of the present invention is the same as SEQ ID NO: 2 attached to the present specification. Thus, Bifidobacterium longum NK49 of the present invention may include the 16S rDNA of SEQ ID NO: 2.

As a result of analyzing said 16S rDNA sequence of SEQ ID NO: 2, this sequence was 99% homologous to that of generally known Bifidobacterium longum strains, thus showing the highest molecular phylogenetic relationship with Bifidobacterium longum. Thus, said lactic acid bacterium was identified as Bifidobacterium longum, then named as Bifidobacterium longum NK49, and then deposited to the KCCM on Aug. 04, 2017 (accession number: KCCM12088P).

The Bifidobacterium longum NK49 of the present invention is a gram-positive bacterium and a cell form thereof is bacillus. More particularly, the physiological characteristics of Bifidobacterium longum NK49 may be analyzed according to a conventional method in the art, and the results thereof are as shown in a following table 3. Particularly, Bifidobacterium longum NK49 may use the following as a carbon source: L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, mannitol, sorbitol, α-methyl-D-glucoside, esculin, salicin, maltose, lactose, melibiose, sucrose, raffinose and D-turanose.

In still another aspect, the present invention provides a pharmaceutical composition for preventing or treating inflammatory diseases, containing Lactobacillus plantarum NK3 KCCM12089P, Bifidobacterium longum NK49 KCCM12088P or a mixture thereof.

The “Lactobacillus plantarum NK3” of the present invention is the same as described above.

Particularly, the Lactobacillus plantarum NK3 contained in the pharmaceutical composition of the present invention may be a live bacterial cell thereof, a dead bacterial cell thereof, a culture product thereof, a crushed product thereof or an extract thereof, but any type of Lactobacillus plantarum NK3 may be used without limitation, as long as it may achieve a preventive or therapeutic effect on

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inflammatory diseases.

The "Bifidobacterium longum NK49" of the present invention is the same as described above.

Particularly, the Bifidobacterium longum NK49 contained in the pharmaceutical composition of the present invention may be a live bacterial cell thereof, a dead bacterial cell thereof, a culture product thereof, a crushed product thereof or an extract thereof, but any type of Lactobacillus plantarum NK3 may be used without limitation, as long as it may achieve a preventive or therapeutic effect on inflammatory diseases.

In the present invention, the term "culture product" means an object obtained by culturing a lactic acid bacterium in a generally known liquid medium or solid medium, and is a concept encompassing a novel lactic acid bacterium in the present invention.

An inflammatory disease of the present invention may be at least one selected from the group including arthritis, gout, hepatitis, asthma, obesity, corneitis, gastritis, enteritis, nephritis, colitis, diabetes, tuberculosis, bronchitis, pleurisy, peritonitis, spondylitis, pancreatitis, inflammatory pain, urethritis, cystitis, vaginitis, arteriosclerosis, septicemia, burn, dermatitis, periodontitis and gingivitis.

In one embodiment of the present invention, it was identified that an inflammatory reaction is remarkably inhibited when macrophage isolated from a mouse is treated with Lactobacillus plantarum NK3 or Bifidobacterium longum NK49 along with lipopolysaccharide, which is an inflammatory reaction inducer (FIGS. 1 and 2). From the results, it was identified that the pharmaceutical composition containing said Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a mixture thereof may be valuably used in preventing and treating inflammatory diseases.

Particularly, said inflammatory disease may be vaginitis.

In one embodiment of the present invention, it was identified that Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a mixture thereof shows an effect of inhibiting the growth of Gardnerella vaginalis and Atopobium vaginae, which are vaginitis-inducing bacteria, and has an

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excellent effect of inhibiting infection with said Gardnerella vaginalis (FIGS. 3 to 5). Also, in one embodiment of the present invention, as a result of administering Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a mixture thereof into an animal model with induced vaginitis, it was identified that inflammations with edema caused by vaginitis bacteria and the number of vaginitis bacteria are decreased, an activity of myeloperoxidase, which is a representative inflammatory indicator in the vagina, is decreased, an expression of TNF-α is increased in the vaginal mucosa, and an expression of IL-10 is increased (FIGS. 6 to 10). From the results, it was identified that the pharmaceutical composition containing the Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a mixture thereof may be valuably used in preventing and treating vaginitis, which is a particular inflammatory disease.

Particularly, said inflammatory disease may be colitis.

In one embodiment of the present invention, as a result of administering Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a mixture thereof into an animal model with induced colitis, it was identified that a length of colon is recovered, an activity of myeloperoxidase is decreased, and the expression level and activity of cytokines, which are an inflammatory indicator, are inhibited (Table 6). From the results, it was identified that the pharmaceutical composition containing the Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a mixture thereof may be valuably used in preventing and treating colitis, which is a particular inflammatory disease.

The pharmaceutical composition according to the present invention may be prepared into a pharmaceutical dosage form by a well-known method in the art, so that an active component of the composition may be provided via a fast, suspended or prolonged release, after being administered into a mammal. When preparing a dosage form, the pharmaceutical composition according to the present invention may further contain a pharmaceutically acceptable carrier, to the extent that this carrier does not suppress an activity of a novel lactic acid bacterium.

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Said pharmaceutically acceptable carrier may include conventionally used ones, for example, lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starch, acacia rubber, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methyl cellulose, microcrystalline cellulose, polyvinyl pyrrolidone, water, methyl hydroxybenzoate, propylhydroxybenzoate, talc, magnesium stearate, mineral oil and the like, but is not limited thereto. Also, the pharmaceutical composition of the present invention may contain a diluent or an excipient such as filler, extender, binder, humectant, disintegrant, surfactant, etc., or other pharmaceutically acceptable additives.

A dosage of the pharmaceutical composition according to the present invention needs to be a pharmaceutically effective amount. The "pharmaceutically effective amount" means an amount enough to prevent or treat inflammatory diseases at a reasonable benefit/risk ratio applicable to medical treatment. An effective dose level may be variously selected by those skilled in the art according to factors such as a formulation method, a patient's condition and weight, the patient's gender, age and degree of disease, a drug form, an administration route and period, an excretion rate, reaction sensitivity, etc. The effective amount may vary depending on a route of treatment, a use of excipients and a possibility of being used with other drugs, as recognized by those skilled in the art. However, in case of a preparation for oral administration to achieve a preferable effect, the composition of the present invention may be generally administered into an adult in an amount of 0.0001 to 100 mg/kg a day, preferably 0.001 to 100 mg/kg a day based on 1 kg of body weight. When the preparation is administered as above, Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a mixture thereof according to the present invention may be administered in an amount of 1×10^2 CFU/kg to 1×10^{11} CFU/kg a day. This administration may be done once a day or several times a day by dividing the preparation. Said dosage does not limit the scope of the present invention in any aspect.

The pharmaceutical composition of the present invention may be administered to mammals such as mice, livestock, humans, etc. through various routes. Particularly, the pharmaceutical

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composition of the present invention may be orally or parenterally administered (for example, applied or injected intravenously, subcutaneously or intraperitoneally), but may be preferably orally administered. The pharmaceutical composition may be intravaginally administered to prevent and treat vaginitis. A solid preparation for oral administration may include powder, granule, tablet, capsule, soft capsule, pill, etc. A liquid preparation for oral administration may include a suspending agent, liquid for internal use, emulsion, syrup, aerosol, etc., but may also include various excipients, for example, humectant, sweetening agent, flavoring agent, preservative, etc. in addition to water and liquid paraffin, which are frequently used simple diluents. A preparation for parenteral administration may be used by being formulated into a dosage form of external preparation and sterilized injectable preparation such as sterilized aqueous solution, liquid, non-aqueous solvent, suspending agent, emulsion, eye drop, eye ointment, syrup, suppository, aerosol, etc., according to respective conventional methods, and preferably may be used by preparing a pharmaceutical composition of cream, gel, patch, spray, ointment, plaster, lotion, liniment, eye ointment, eye drop, paste or cataplasma, but is not limited thereto. A preparation for local administration may be an anhydrous or aqueous form depending on a clinical prescription. As the non-aqueous solvent and the suspending agent above, propylene glycol, polyethylene glycol, vegetable oil like olive oil, injectable ester like ethyl oleate, etc. may be used. As a base of the suppository, the following may be used: witepsol, macrogol, tween 61, cacao butter, laurinum, glycerogelatin, etc.

In still another aspect, the present invention provides a method for preventing or treating inflammatory diseases, including administering Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a mixture thereof into a subject.

In the present invention, the terms “Lactobacillus plantarum NK3,” “Bifidobacterium longum NK49,” “administration,” “inflammatory disease” and the like are the same as described above.

Said subject refers to an animal, and may be typically a mammal, on which treatment using

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the novel lactic acid bacteria of the present invention may show a beneficial effect. A preferable example of this subject may include primates like humans. Also, these subjects may include all the subjects having a symptom of inflammatory diseases, or having a risk of having the symptom.

In still another aspect, the present invention provides a health functional food for preventing or ameliorating inflammatory diseases, containing Lactobacillus plantarum NK3 KCCM12089P, Bifidobacterium longum NK49 KCCM12088P or a mixture thereof.

In the present invention, the terms "Lactobacillus plantarum NK3," "Bifidobacterium longum NK49," "administration," "inflammatory disease" and the like are the same as described above.

The health functional food, which puts an emphasis on a body modulating function of food, is a food, which is given value added to work and express for a particular purpose by using a physical, biochemical or bioengineering method. A component of this health functional food is designed and processed to fully exert a body modulating function in vivo, which is involved in defending a living body, adjusting a body rhythm, preventing a disease and recovering from the disease, and may contain food supplementary additives, sweeteners or functional raw materials, which are acceptable as food.

In case of using Lactobacillus plantarum NK3 or Bifidobacterium longum NK49 of the present invention as a health functional food (or health functional beverage additives), said novel lactic acid bacteria may be added thereto as they are, used along with other food or food ingredients, or appropriately used according to a conventional method. A mixed amount of said Lactobacillus plantarum NK3 or Bifidobacterium longum NK49 may be appropriately determined depending on a purpose of use thereof (prevention, health, improvement or therapeutic treatment).

Said health functional food may contain various nutrients, vitamins, minerals (electrolytes), flavoring agents such as synthetic flavoring agents, natural flavoring agents and the like, coloring agents and enhancers (cheese, chocolate, etc.), pectic acid and salts thereof, organic acid, protective colloidal thickeners, pH adjusting agents, stabilizers, preservatives, glycerin, alcohol, carbonators used in

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carbonated beverages, etc. Also, the health functional food of the present invention may contain pulp for preparing fruit and vegetable based beverages. These components may be used alone or in combination, and a ratio of the additives is generally selected from a range of 0.001 to 50 parts by weight with regard to the total weight of the composition.

A type of said health functional food has no particular limitation. Food, to which said Lactobacillus plantarum NK3 or Bifidobacterium longum NK49 may be added, includes sausage, meats, bread, chocolates, snacks, candies, confectionery, ramen, pizza, other noodles, chewing gums, dairy products including ice creams, various soups, beverages, teas, health drinks, alcohol beverages, vitamin complexes and the like. In case of being formulated into beverages, a liquid component, which is added to the beverages in addition to the novel lactic acid bacteria, may include various flavors, natural carbohydrates or the like as an additional component just as contained in conventional beverages, but is not limited thereto. The natural carbohydrates mentioned above may be monosaccharide (ex. glucose, fructose, etc.), disaccharide (ex. maltose, sucrose, etc.) and polysaccharide (ex. conventional sugar such as dextrin, cyclodextrin, etc.), as well as sugar alcohol such as xylitol, sorbitol, erythritol, etc.

In still another aspect, the present invention provides a use of Lactobacillus plantarum NK3 KCCM12089P, Bifidobacterium longum NK49 KCCM12088P or a mixture thereof for preparing a drug for treating inflammatory diseases.

In still another aspect, the present invention provides a composition containing Lactobacillus plantarum NK3 KCCM12089P, Bifidobacterium longum NK49 KCCM12088P or a mixture thereof for a use in treating inflammatory diseases.

In still another aspect, the present invention provides a use of Lactobacillus plantarum NK3 KCCM12089P, Bifidobacterium longum NK49 KCCM12088P or a mixture thereof for treating inflammatory diseases.

The numerical values described in the present specification as above should be interpreted to

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include a range of equivalents thereof, unless otherwise stated.

Hereinafter, the present invention will be described in detail through preferred Examples for better understanding of the present invention. However, the following Examples are provided only for the purpose of illustrating the present invention, and thus the present invention is not limited thereto.

Advantageous Effects

Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a mixture thereof, which is a novel lactic acid bacterium according to the present invention, has an effect of inhibiting inflammatory reactions. Thus, the novel lactic acid bacteria according to the present invention may be used as a composition for preventing or treating inflammatory diseases, and particularly effective in treating and preventing colitis and vaginitis.

Brief Description of the Drawings

FIG. 1 is a graph showing inhibitory capacity of Lactobacillus plantarum NK3 and Bifidobacterium longum NK49, which are novel lactic acid bacteria, against an activity of NF-kB (p-p65/p65) to macrophage.

FIG. 2 is a graph showing inhibitory capacity of Lactobacillus plantarum NK3 and Bifidobacterium longum NK49, which are novel lactic acid bacteria, against an expression level of TNF-α to macrophage.

FIG. 3 is a graph showing an antibacterial effect of Lactobacillus plantarum NK3 and Bifidobacterium longum NK49, which are novel lactic acid bacteria, against Gardnerella vaginalis.

FIG. 4 is a graph showing an antibacterial effect of Lactobacillus plantarum NK3 and Bifidobacterium longum NK49, which are novel lactic acid bacteria, against Atopobium vaginae.

FIG. 5 is a graph showing an inhibitory capacity of Lactobacillus plantarum NK3 and Bifidobacterium longum NK49, which are novel lactic acid bacteria, against infection with Gardnerella vaginalis.

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G: Group treated with Gardnerella vaginalis only; GL5, GL6, GL7: Group treated with Gardnerella vaginalis and then treated with Lactobacillus plantarum at $1x10^6$ CFU/mL, $1x10^7$ CFU/mL and $1x10^8$ CFU/mL respectively; and GB5, GB6, GB7: Group treated with Gardnerella vaginalis and then treated with Bifidobacterium longum at $1x10^6$ CFU/mL, $1x10^7$ CFU/mL and $1x10^8$ CFU/mL respectively.

FIG. 6 is a view showing an identified effect of Lactobacillus plantarum NK3, Bifidobacterium longum NK49 and a mixture thereof, which are novel lactic acid bacteria, on inhibiting inflammations in the vagina and the uterus.

N: Normal group; GC: Group infected with Gardnerella vaginalis only; oGL, oGB, oGM: Group of experimental animals infected with Gardnerella vaginalis, orally dosed with respective Lactobacillus plantarum, Bifidobacterium longum or a mixture thereof; and vGL, vGB, vGM: Group of experimental animals infected with Gardnerella vaginalis, intravaginally dosed with respective Lactobacillus plantarum, Bifidobacterium longum or a mixture thereof. Hereinafter the same.

FIG. 7 is a graph showing an inhibitory capacity of Lactobacillus plantarum NK3, Bifidobacterium longum NK49 and a mixture thereof, which are novel lactic acid bacteria, against infection with Gardnerella vaginalis.

FIG. 8 is a graph showing an inhibitory capacity of Lactobacillus plantarum NK3, Bifidobacterium longum NK49 and a mixture thereof, which are novel lactic acid bacteria, against an activity of myeloperoxidase.

FIG. 9 is a graph showing an inhibitory capacity of Lactobacillus plantarum NK3, Bifidobacterium longum NK49 and a mixture thereof, which are novel lactic acid bacteria, against an expression of TNF-α.

FIG. 10 is a graph showing an increasing capacity of Lactobacillus plantarum NK3, Bifidobacterium longum NK49 and a mixture thereof, which are novel lactic acid bacteria, for an

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expression of IL-10.

FIG. 11 is a graph showing an effect of Lactobacillus plantarum NK3, Bifidobacterium longum NK49 and a mixture thereof, which are novel lactic acid bacteria, on recovering Lactobacilli in the vagina.

Mode for Invention

Hereinafter, the present invention will be described in detail through preferred Examples for better understanding of the present invention. However, the following Examples are provided only for the purpose of illustrating the present invention, and thus the present invention is not limited thereto.

Example 1: Isolation and identification of lactic acid bacteria

(1) Isolation of lactic acid bacteria from human feces

Human feces were inserted and suspended in GAM liquid medium (GAM broth; Nissui Pharmaceutical, Japan). After that, the supernatant was taken and transplanted into BL agar medium (Nissui Pharmaceutical, Japan), and then anaerobically cultured at 37°C for about 48 hours, so as to isolate colony-forming strains therefrom.

(2) Isolation of lactic acid bacteria from kimchi

Cabbage kimchi, radish kimchi or green onion kimchi was crushed respectively, after which crushed supernatant was taken and transplanted into MRS agar medium (Difco, USA), and then anaerobically cultured at 37°C for about 48 hours, so as to isolate colony-forming strains therefrom.

(3) Identification of isolated lactic acid bacteria

Physiological characteristics and 16S rDNA sequences of the strains isolated from human feces or kimchi were analyzed to identify species of the strains, and then names were given to the strains. Strain names given to lactic acid bacteria are the same as shown in the following table 1. Particularly, the lactic acid bacteria isolated from kimchi were five species of Lactobacillus plantarum (management nos. 1 to 5 of Table 1), five species of Lactobacillus brevis (management nos. 6 to 10 of Table 1), five species of Lactobacillus sakei (management nos. 11 to 15 of Table 1), and five species of Lactobacillus

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curvatus (management nos. 16 to 20 of Table 1). The lactic acid bacteria isolated from human feces were five species of Lactobacillus rhamnosus (management nos. 21 to 25 of Table 1), five species of Lactobacillus plantarum (management nos. 26 to 30 of Table 1), five species of Lactobacillus reuteri (management nos. 31 to 35 of Table 1), four species of Lactobacillus johnsonii (management nos. 36 to 39 of Table 1), three species of Lactobacillus mucosae (management nos. 40 to 42 of Table 1), three species of Bifidobacterium adolescentis (management nos. 43 to 45 of Table 1), and five species of Bifidobacterium longum (management nos. 46 to 50 of Table 1).

【Table 1】

Management no.	Strain name	Management no.	Strain name
1	Lactobacillus plantarum NK1	26	Lactobacillus plantarum NK26
2	Lactobacillus plantarum NK2	27	Lactobacillus plantarum NK27
3	Lactobacillus plantarum NK3	28	Lactobacillus plantarum NK28
4	Lactobacillus plantarum NK4	29	Lactobacillus plantarum NK29
5	Lactobacillus plantarum NK5	30	Lactobacillus plantarum NK30
6	Lactobacillus brevis NK6	31	Lactobacillus reuteri NK31
7	Lactobacillus brevis NK7	32	Lactobacillus reuteri NK32
8	Lactobacillus brevis NK8	33	Lactobacillus reuteri NK33
9	Lactobacillus brevis NK9	34	Lactobacillus reuteri NK34
10	Lactobacillus brevis NK10	35	Lactobacillus reuteri NK35
11	Lactobacillus sakei NK11	36	Lactobacillus johnsonii NK36
12	Lactobacillus sakei NK12	37	Lactobacillus johnsonii NK37
13	Lactobacillus sakei NK13	38	Lactobacillus johnsonii NK38
14	Lactobacillus sakei NK14	39	Lactobacillus johnsonii NK39
15	Lactobacillus sakei NK15	40	Lactobacillus mucosae NK40
16	Lactobacillus curvatus NK16	41	Lactobacillus mucosae NK41
17	Lactobacillus curvatus NK17	42	Lactobacillus mucosae NK42
18	Lactobacillus curvatus NK18	43	Bifidobacterium adolescentis NK43
19	Lactobacillus curvatus NK19	44	Bifidobacterium adolescentis NK44
20	Lactobacillus curvatus NK20	45	Bifidobacterium adolescentis NK45
21	Lactobacillus rhamnosus NK21	46	Bifidobacterium longum NK46
22	Lactobacillus rhamnosus NK22	47	Bifidobacterium longum NK47
23	Lactobacillus rhamnosus NK23	48	Bifidobacterium longum NK48
24	Lactobacillus rhamnosus NK24	49	Bifidobacterium longum NK49
25	Lactobacillus rhamnosus	50	Bifidobacterium longum NK50

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	NK25		

(4) Physiological characteristics of novel lactic acid bacterium Lactobacillus plantarum NK3

Out of strains described in Table 1 above, it was identified that Lactobacillus plantarum NK3 (accession number KCCM12089P) is a gram-positive bacillus. Also, it was shown that 16S rDNA of Lactobacillus plantarum NK3 has a sequence of SEQ ID NO: 1. As a result of comparing the 16S rDNA sequence of Lactobacillus plantarum NK3 through BLAST search, it was shown that a Lactobacillus plantarum strain having the same 16S rDNA sequence is not searched at all, and the sequence was 99% homologous to the 16S rDNA sequence of a generally known Lactobacillus plantarum strain.

Out of physiological characteristics of Lactobacillus plantarum NK3, availability of carbon source was analyzed with a sugar fermentation test using API 50 CHL kit. The results thereof are the same as shown in a following table 2. In Table 2 below, "+" indicates that the availability of carbon source is positive and "-" indicates that the availability of carbon source is negative.

【Table 2】

Carbon source	NK3	Carbon source	NK3
CONTROL	-	Esculin	+
Glycerol	-	Salicin	+
Erythritol	-	Cellobiose	+
D-arabinose	-	Maltose	+
L-arabinose	+	Lactose	+
D-ribose	+	Melibiose	+
D-xylose	-	Sucrose	+
L-xylose	-	Trehalose	+
D-adonitol	-	Inulin	-
Methyl-BD-xylopyranoside	-	Melezitose	+
D-galactose	+	Raffinose	-
D-glucose	+	Starch	-
D-fructose	+	Glycogen	-
D-mannose	+	Xylitol	-
L-sorbose	-	Gentiobiose	+
Rhamnosus	-	D-turanose	+

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Dulcitol	-	D-lyxose	-
Inositol	-	D-tagatose	-
Mannitol	+	D-fucose	-
Solbitol	+	L-fucose	-
α-methyl-D-mannoside	±	D-arabitol	-
α-methyl-D-glucoside	-	L-arabitol	-
N-acetyl-glucosamine	+	Gluconate	±
Amygdalin	+	2-keto-gluconate	-
Arbutin	+	5-keto-gluconate	-

(5) Physiological characteristics of novel lactic acid bacterium Bifidobacterium longum NK49

Out of strains described in Table 1 above, it was identified that Bifidobacterium longum NK49 (accession number KCCM12088P) is a gram-positive bacillus. Also, it was shown that 16S rDNA of Bifidobacterium longum NK49 has a sequence of SEQ ID NO: 2. As a result of comparing the 16S rDNA sequence of Bifidobacterium longum NK49 through BLAST search, it was shown that a Bifidobacterium longum strain having the same 16S rDNA sequence is not searched at all, and 99% homologous to the 16S rDNA sequence of a generally known Bifidobacterium longum strain.

Out of physiological characteristics of Bifidobacterium longum NK49, availability of carbon source was analyzed with a sugar fermentation test using API 50 CHL kit. The results thereof are the same as shown in a following table 3. In Table 3 below, "+" indicates that the availability of carbon source is positive and "-" indicates that the availability of carbon source is negative.

【Table 3】

Carbon source	NK49	Carbon source	NK49
CONTROL	-	Esculin	+
Glycerol	-	Salicin	+
Erythritol	-	Cellobiose	-
D-arabinose	-	Maltose	+
L-arabinose	+	Lactose	+
D-ribose	+	Melibiose	+
D-xylose	±	Sucrose	+
L-xylose	-	Trehalose	-

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D-adonitol	-	Inulin	-
Methyl-BD-xylopyranoside	-	Melezitose	-
D-galactose	+	Raffinose	+
D-glucose	+	Starch	-
D-fructose	+	Glycogen	-
D-mannose	-	Xylitol	-
L-sorbose	-	Gentiobiose	-
Rhamnosus	-	D-turanose	±
Dulcitol	-	D-lyxose	-
Inositol	-	D-tagatose	-
Mannitol	+	D-fucose	-
Sorbitol	+	L-fucose	-
α-methyl-D-mannoside	-	D-arabitol	-
α-methyl-D-glucoside	±	L-arabitol	-
N-acetyl-glucosamine	-	Gluconate	-
Amygdalin	-	2-keto-gluconate	-
Arbutin	-	5-keto-gluconate	-

Example 2: Comparison of activity of isolated lactic acid bacteria

(1) Antioxidant activity (in vitro)

DPPH (2,2-Diphenyl-1-picrylhydrazyl) was dissolved in ethanol to reach a 0.2 mM concentration, such that a DPPH solution was prepared. A suspension of lactic acid bacteria (1×10^6 CFU/mℓ) or a vitamin C solution (1 g/mℓ) was inserted into 0.1 mℓ of said DPPH solution, and then cultured at 37°C for 20 minutes. A culture fluid was centrifuged at 3000 rpm for five minutes, such that supernatant was obtained. After that, an absorbance of the supernatant was measured at 517 nm, and then antioxidant activity of isolated lactic acid bacteria was calculated accordingly. Antioxidant activity for each lactic acid bacterium is the same as shown in a following table 4.

(2) Measurement of inflammatory indicators in macrophage

2 mℓ of sterilized 4% thioglycolate was intraperitoneally administered into a C57BL/6 mouse (male, 6 weeks old and 20-23 g). In 96 hours later, the mouse was anesthetized, and then 8 mℓ of RPMI

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1640 medium was intraperitoneally administered to the mouse. In 5 to 10 minutes later, the RPMI medium (macrophage) was intraperitoneally extracted from the mouse, then centrifuged at 1000 g for 10 minutes, and then washed twice again with RPMI 1640 medium. Said macrophage was plated in a 24-well plate at 0.5×10^6 per well, and then treated with the isolated lactic acid bacteria (final treatment concentration: 1×10^4 cfu/ml, hereinafter the same) and lipopolysaccharide (LPS), which is an inflammatory reaction inducer, for 2 or 24 hours, such that supernatant and cells were obtained therefrom. The obtained cells were inserted into an RIPA buffer (Gibco) and homogenized. An expression level of cytokines such as TNF-α, IL-10, etc. was measured from a culture supernatant treated for 24 hours and expression levels of p65 (NF-κB), p-p65 (phosphor-NF-κB) and β-actin were measured from the cells obtained with treatment for two hours through an immunoblotting method. Expression levels of inflammation indicators for each lactic acid bacterium are the same as shown in a following table 4.

(Criteria when measuring activity in Table 4: +++, >90%, very strong; ++, >60-90%, strong; +, >20-60%, weak; and -, <20%, insignificant effect, the same as Table 5)

【Table 4】

Management no.	Strain name	Antioxidant activity	TNF-α inhibitory capacity	IL-10 expression increase	NF-kB inhibitory capacity
1	Lactobacillus plantarum NK1	+	+	+	+
2	Lactobacillus plantarum NK2	+	++	+	+
3	Lactobacillus plantarum NK3	+++	+++	+++	+++
4	Lactobacillus plantarum NK4	+	+	+	++
5	Lactobacillus plantarum NK5	++	++	++	++
6	Lactobacillus brevis NK6	+	+	+	+
7	Lactobacillus brevis NK7	+	+	+	+
8	Lactobacillus brevis NK8	+	+	+	+
9	Lactobacillus brevis NK9	+	+	+	+
10	Lactobacillus brevis NK10	-	+	+	+
11	Lactobacillus sakei NK11	+	+	+	+
12	Lactobacillus sakei NK12	-	++	+	+

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13	Lactobacillus sakei NK13	-	++	++	+
14	Lactobacillus sakei NK14	-	+	+	+
15	Lactobacillus sakei NK15	+	+	+	+
16	Lactobacillus curvatus NK16	+	+	+	+
17	Lactobacillus curvatus NK17	+	+	+	+
18	Lactobacillus curvatus NK18	+	+	+	+
19	Lactobacillus curvatus NK19	+	+	+	+
20	Lactobacillus curvatus NK20	+	+	+	+
21	Lactobacillus rhamnosus NK21	+	+	+	+
22	Lactobacillus rhamnosus NK22	+	+	+	+
23	Lactobacillus rhamnosus NK23	+	+	+	+
24	Lactobacillus rhamnosus NK24	++	+	+	+
25	Lactobacillus rhamnosus NK25	++	++	++	++
26	Lactobacillus plantarum NK26	+	+	+	+
27	Lactobacillus plantarum NK27	+	+	+	+
28	Lactobacillus plantarum NK28	+	+	+	+
29	Lactobacillus plantarum NK29	+	+	+	+
30	Lactobacillus plantarum NK30	+	+	+	+
31	Lactobacillus reuteri NK31	+	+	+	+
32	Lactobacillus reuteri NK32	++	++	++	++
33	Lactobacillus reuteri NK33	+++	++	++	++
34	Lactobacillus reuteri NK34	+	+	+	+
35	Lactobacillus reuteri NK35	+	+	+	+
36	Lactobacillus johnsonii NK36	++	++	++	+
37	Lactobacillus johnsonii NK37	++	++	++	++
38	Lactobacillus johnsonii NK38	+	+	+	+
39	Lactobacillus johnsonii NK39	+	+	+	+
40	Lactobacillus mucosae NK40	++	++	++	++
41	Lactobacillus mucosae NK41	++	++	+++	+++
42	Lactobacillus mucosae NK42	+	+	+	+
43	Bifidobacterium adolescentis NK43	+	+	+	+
44	Bifidobacterium adolescentis NK44	++	++	++	++
45	Bifidobacterium adolescentis NK45	+	+	+	+
46	Bifidobacterium longum NK46	+++	++	+++	+++
47	Bifidobacterium longum NK47	++	++	++	++
48	Bifidobacterium longum NK48	+	+	+	+
49	Bifidobacterium longum NK49	+++	+++	+++	+++
50	Bifidobacterium longum NK50	+	+	+	+

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(3) Effect on ZO-1 protein expression in Caco2 cells

Caco2 cells, which are colon cancer cells, were purchased from the Korean Cell Line Bank, then cultured in RPMI 1640 medium for 48 hours, and then were seeded in a 12-well plate at 2×10^6 cells per well. Each well was treated with 1 μg of LPS only, or treated with 1 μg of LPS and 1×10^4 CFU of lactic acid bacteria together, and then cultured for 24 hours. After that, the cultured cells were collected from each well, and an expression level of ZO-1, which is a tight junction protein, was measured by an immunoblotting method. Expression levels of ZO-1 for each lactic acid bacterium are the same as shown in a following table 5.

【Table 5】

Management no.	Strain name	ZO-1 Expression increase
1	Lactobacillus plantarum NK1	+
2	Lactobacillus plantarum NK2	+
3	Lactobacillus plantarum NK3	++
4	Lactobacillus plantarum NK4	+
5	Lactobacillus plantarum NK5	++
6	Lactobacillus brevis NK6	+
7	Lactobacillus brevis NK7	+
8	Lactobacillus brevis NK8	+
9	Lactobacillus brevis NK9	+
10	Lactobacillus brevis NK10	-
11	Lactobacillus sakei NK11	+
12	Lactobacillus sakei NK12	-
13	Lactobacillus sakei NK13	+
14	Lactobacillus sakei NK14	+
15	Lactobacillus sakei NK15	+
16	Lactobacillus curvatus NK16	+
17	Lactobacillus curvatus NK17	+
18	Lactobacillus curvatus NK18	+
19	Lactobacillus curvatus NK19	+
20	Lactobacillus curvatus NK20	+
21	Lactobacillus rhamnosus NK21	+
22	Lactobacillus rhamnosus NK22	+

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23	Lactobacillus rhamnosus NK23	+
24	Lactobacillus rhamnosus NK24	++
25	Lactobacillus rhamnosus NK25	+
26	Lactobacillus plantarum NK26	+
27	Lactobacillus plantarum NK27	+
28	Lactobacillus plantarum NK28	+
29	Lactobacillus plantarum NK29	+
30	Lactobacillus plantarum NK30	+
31	Lactobacillus reuteri NK31	+
32	Lactobacillus reuteri NK32	++
33	Lactobacillus reuteri NK33	++
34	Lactobacillus reuteri NK34	+
35	Lactobacillus reuteri NK35	+
36	Lactobacillus johnsonii NK36	+
37	Lactobacillus johnsonii NK37	++
38	Lactobacillus johnsonii NK38	+
39	Lactobacillus johnsonii NK39	+
40	Lactobacillus mucosae NK40	++
41	Lactobacillus mucosae NK41	++
42	Lactobacillus mucosae NK42	+
43	Bifidobacterium adolescentis NK43	+
44	Bifidobacterium adolescentis NK44	++
45	Bifidobacterium adolescentis NK45	+
46	Bifidobacterium longum NK46	++
47	Bifidobacterium longum NK47	+
48	Bifidobacterium longum NK48	+
49	Bifidobacterium longum NK49	+
50	Bifidobacterium longum NK50	+

(5) Experimental results

As a result of evaluating an activity of isolated lactic acid bacteria, it was identified that Lactobacillus plantarum NK3 and Bifidobacterium longum NK49, which are novel lactic bacteria out of the isolated lactic acid bacteria, increase an expression level of ZO-1, which is a tight junction protein, thereby showing an excellent antioxidant activity and an excellent effect of inhibiting inflammatory reactions (Tables 4 and 5).

Example 3: Measurement of inhibitory capacity against inflammatory reactions of

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macrophage

In Example 2 above, it was identified that there is an effect of inhibiting inflammatory reactions depending on a concentration of administration of Lactobacillus plantarum NK3 and Bifidobacterium longum NK49, which are novel lactic acid bacteria having excellent antioxidant activity and an excellent effect of inhibiting inflammatory reactions.

Particularly, 2 mℓ of sterilized 4% thioglycolate was intraperitoneally administered into a C57BL/6 mouse (male, 20-23 g). In 96 hours later, the mouse was anesthetized, and then 8 mℓ of RPMI 1640 medium was intraperitoneally administered into the mouse. In 5 to 10 minutes later, the RPMI medium (macrophage) was intraperitoneally extracted from the mouse, then centrifuged at 1000 g for 10 minutes, and then washed twice again with RPMI 1640 medium. After being cultured in a 24-well plate for five hours, attached cells were used as macrophage. The macrophage was plated at 0.5×10^6 per well, and treated with 10^3, 10^4 and 10^5 CFU/mL of Lactobacillus plantarum NK3 and Bifidobacterium longum NK49, which are novel lactic acid bacteria, as well as lipopolysaccharide (LPS), which is an inflammatory reaction inducer, for 90 minutes or 24 hours, such that supernatant and cells were obtained therefrom. The obtained cells were inserted into an RIPA buffer (Gibco) and homogenized. Expression levels of p65 (NF-κB), p-p65 (phosphor-NF-kB) and β-actin were measured from the cells obtained with treatment for 90 minutes through an immunoblotting method. An expression level of TNF-α cytokines was measured by ELISA kit (Ebioscience, San Diego, CA, USA) from the culture supernatant obtained after treatment for 24 hours.

In result, it was identified that an activity of NF-kB is inhibited and an expression level of TNF-α is inhibited in all the groups treated with Lactobacillus plantarum NK3 or Bifidobacterium longum NK49 (FIGS. 1 and 2).

Example 4: Antibacterial activity against vaginitis-causing bacteria

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(1) Antibacterial test of lactic acid bacteria

Lactobacillus plantarum NK3 and Bifidobacterium longum NK49 ($1x10^6$, $1x10^7$, $1x10^8$ CFU/mL), which were the isolated novel lactic acid bacteria, were transplanted into GAM medium along with Gardnerella vaginalis or Atopobium vaginae ($1x10^6$ CFU/mL). Culture was performed for 24 hours at 37°C under an anaerobic condition in BHIS medium, in which yeast extract (1%), maltose (0.1%), glucose (0.1%) and horse serum (10%) were added into BHI broth, such that antibacterial activity was measured.

In result, it was identified that both Lactobacillus plantarum NK3 and Bifidobacterium longum NK49 show an effect of inhibiting growth of Gardnerella vaginalis and Atopobium vaginae by 95% or more (FIGS. 3 and 4).

(2) Infection inhibitory capacity of lactic acid bacteria

While HeLa cells, which are a human cervical cancer cell line, were cultured in RPMI 1640 medium (containing 10% heat-inactivated fetal calf serum) at 37°C, 5% CO_2 and 95% air conditions, Lactobacillus plantarum NK3 and Bifidobacterium longum NK49 ($1x10^4$, $1x10^6$ CFU/mL), which were the isolated lactic acid bacteria, were transplanted thereinto alone or in combination with said lactic acid bacteria ($1x10^5$, $1x10^6$, $1x10^7$ CFU/mL) as well as Gardnerella vaginalis ($1x10^5$ CFU/mL), such that the number of bacteria attached to the HeLa cells was measured through qPCR in 24 hours later.

In result, it was identified that the number of Gardnerella vaginalis attached is remarkably decreased in the HeLa cells, into which Lactobacillus plantarum NK3 or Bifidobacterium longum NK49 was transplanted together (FIG. 5).

From the above experimental results, it can be understood that the novel lactic acid bacteria show not only an effect of inhibiting the growth of vaginitis-inducing bacteria, but also an effect of inhibiting infection with bacteria.

Example 5: Effect of lactic acid bacteria on treating vaginitis in animal model

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(1) Preparation of animal model with vaginitis and administration of lactic acid bacteria

An experiment was performed by using six C57BL/6 mice (female, 19-22 g and 6 weeks old) per group. 0.125 mg of β-estradiol 17-benzoate (Sigma Inc., MO, USA) was dissolved in olive oil and subcutaneously injected into each of said mice. Three days later, Gardnerella vaginalis (1x 10^8 CFU/mouse) was transplanted into the vagina of the mouse. Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a 1:1 mixture thereof, which was the isolated novel lactic acid bacteria, was administered orally into the mouse or directly into the vagina thereof at 1x10^9 CFU/mouse once every day for 14 days from the 8th day after transplantation. The last administration was performed on the 7th day after the administration of lactic acid bacteria (14th day after transplantation), and an animal model was sacrificed in 24 hours later to perform an experiment.

(2) Identification of occurrence of intravaginal inflammations

As a result of transplanting Gardnerella vaginalis into the vagina of the mouse as above, an inflammation with edema occurred to the vagina and the uterus. However, as a result of orally or intravaginally administering Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a mixture thereof, which is a novel lactic acid bacterium, it was identified that inflammations are significantly decreased in appearance in the vagina and the uterus (FIG. 6).

(3) Quantification of lactic acid bacteria and Gardnerella vaginalis strains

After administering the novel lactic acid bacteria as above, the inside of the vagina was washed with 0.5 ml of sterilized saline solution in 24 and 48 hours later. A resultant vaginal wash liquid was isolated with Qaigen DNA purification kit, after which lactic acid bacteria and Gardnerella vaginalis strains were quantified through PCR.

In result, it was identified that the number of infectious Gardnerella vaginalis bacteria is decreased by 99% or more in the group orally or intravaginally dosed with Lactobacillus plantarum NK3,

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Bifidobacterium longum NK49 or a mixture thereof (FIG. 7).

(4) Measurement of myeloperoxidase activity

A vaginal wash liquid obtained as above was added into 1 mL of 50 mM phosphate buffer (pH 6.0) and subjected to supersonic treatment. A process of thawing and freezing was performed three times, followed by centrifugation. 398 μL of o-dianisidine (0.129 mg/mL) was added into 100 μL of supernatant. A final concentration of H_2O_2 was set to 0.0005%, after which activity of myeloperoxidase (MPO) was measured at 25°C and at 492 nm during time course.

In result, in case of the group infected with Gardnerella vaginalis, the activity of myeloperoxidase, which is a representative inflammation indicator of the vagina, was significantly increased. In case of the group orally or intravaginally dosed with Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a mixture thereof, however, it was identified that the activity of myeloperoxidase is remarkably decreased (FIG. 8).

(5) Analysis of transcription factors and cytokines in vaginal mucosa

Lysis buffer (20 mM HEPES, 1.5 mM MgCl2, 0.4 mM NaCl, 1 mM EDTA, 1 mM dithiotheitol, 0.5 mM phenyl methyl sulfonyl fluoride, 20 μg/mL trypsin inhibitor, 1% nonidet p-40, 20% glycerol) was injected into the colon or vaginal mucosa tissues of the animal model sacrificed above and was homogenized. A specimen was prepared to contain 50 μg/μL of proteins by quantifying the proteins with Bradford assay. 10 μg/μL of electrophoretic sample buffer (Laemmli sample buffer 950 μL+ βMe 50 μL) was added into the specimen and denatured at 100°C for three minutes to carry out electrophoresis (150 V, 30 mA). The electrophoresed gel was transferred to a membrane at 30 V for two hours. Said membrane was inserted into 5% skim milk/PBS-T (0.05% tween 20/PBS) in an amount of 15 ml and shaken for one hour. 10 mL of 1% skim milk/PBS-T was inserted into 10 μg/μL of primary antibodies, then subjected to reaction for 12 hours, and then washed with PBS-T solution for five minutes three times. And, 20 mL of 1% skim milk/PBS-T was inserted into 10 μg/μL of secondary

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antibodies with regard to each of cytokines and transcription factors, and then subjected to reaction by shaking for one hour. Washing was performed with PBS-T solution for five minutes three times and ECL solution was subjected to luminescence at last.

In result, in case of the group infected with Gardnerella vaginalis, an expression of TNF-α was increased and an expression of IL-10 was decreased in vaginal tissues. However, in case of the group orally or intravaginally dosed with Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a mixture thereof, it was identified that an expression of TNF-α is inhibited and an expression of IL-10 is increased (FIGS. 9 and 10).

(6) Increase in intravaginal lactic acid bacteria

The inside of the vagina of the mouse was washed twice with 0.5 ml of sterilized saline solution. A resultant vaginal wash liquid was collected and centrifuged (10,000 g, 10 minutes), and DNA was extracted from precipitates by using a bacterial genomic DNA extraction kit (QIAGEN DNeasy Feces kit; Qiagen, Hilden, Germany). 10 ng of the extracted DNA was inserted into Qiagen thermal cycler and the following primers and SYBER premix were inserted thereinto, after which PCR was performed by carrying out DNA polymerase activation (95°C, 30 seconds), denaturation (95°C, 5 seconds) and amplification (63°C, 30 seconds) 38 times.

Lactobacilli forward (5'-3') CTC AAA ACT AAA CAA AGT TTC (SEQ ID NO: 3);

Lactobacilli reverse (5'-3') CTT GTA CAC ACC GCC CGT (SEQ ID NO: 4);

Control 16S rDNA forward (5'-3') AGA GTT TGA TCC TGG CTC AG (SEQ ID NO: 5); and

Control 16S rDNA reverse (5'-3') AAG GAG GTG WTC CAR CC (SEQ ID NO: 6).

In result, in case of being infected with Gardnerella vaginalis, it was identified that Lactobacilli are remarkably decreased. On the other hand, in case of being orally or intravaginally dosed with Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a mixture thereof, it was identified that said Lactobacilli are increased (FIG. 11). It means that Lactobacilli, which are decreased due to vaginitis

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induced by Gardnerella vaginalis, are recovered by the administration of said lactic acid bacteria, so as to restore acidic conditions inside the vagina.

From the above experimental results, it was identified that the novel lactic acid bacteria have an effect on preventing and treating vaginitis.

Example 5: Therapeutic effect of lactic acid bacteria on colitis in animal model

(1) Preparation of animal model with colitis and administration of lactic acid bacteria

An experiment was performed by using six C57BL/6 mice (male, 21-23 g and 6 weeks old) per group after being acclimated in a laboratory for one week. One group was a normal group and the mice in the rest of the groups were induced to develop colitis with 2,4,6-trinitrobenzenesulfonic acid (TNBS). Particularly, the experimental animals were anesthetized with ether, after which 0.1㎖ of TNBS solution mixed in 50% ethanol was rectally administered into each colon of animals by using a 1㎖ syringe with a round top, and then the animals were vertically lifted and maintained for 30 seconds to induce inflammations therefrom. On the other hand, the normal group was orally dosed with 0.1㎖ of saline solution. Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a 1:1 mixture thereof, which is a novel lactic acid bacterium, was suspended in saline solution and orally administered in an amount of $1x10^9$ CFU once daily for three days starting from the following day after administration. On the next day after finishing the administration of lactic acid bacteria, the experimental animals were sacrificed, after which a colon ranging from appendix to a region right before anus was removed from a large intestine and a length thereof was measured. After that, the following various indicators were identified from the above. On the other hand, the experimental animals of the normal group were orally dosed with 1% dextrose solution, which was suspension of lactic acid bacteria, instead of the novel lactic acid bacteria. Also, the experimental animals of the positive control group were orally dosed with 50

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mg/kg of sulfasalazine, which was a therapeutic drug for colitis, instead of the novel lactic acid bacteria.

(2) Measurement of myeloperoxidase activity

200 μℓ of 10 mM potassium phosphate buffer (pH 7.0) containing 0.5% hexadecyl trimethyl ammonium bromide was put into 100 mg of colon tissues, and subjected to homogenization. A resulting product was centrifuged at 4°C and at 10,000 g for 10 minutes, so as to obtain a supernatant therefrom. 50 μℓ of the supernatant was put into 0.95 mℓ of a reaction solution (containing 1.6 mM tetramethyl benzidine and 0.1 mM H_2O_2), and then subjected to reaction at 37°C so as to microscopically measure an observance at 650 nm. An activity of said myeloperoxidase was calculated with a resulting reactant H_2O_2 1 μmol/ml = 1 unit.

(3) Measurement of inflammatory indicators

Inflammatory reaction indicator materials such as p-p65, p65, COX-2 and β-actin were measured by using a western blotting method. Particularly, 50 μg of supernatant, which had been obtained by the same method as shown in an experiment for measuring the activity of said myeloperoxidase (MPO), was taken and subjected to immunoblotting. Also, an expression level of cytokines (IL-17 and TNF-α) was measured by using ELISA kit.

(4) Experimental results

The experimental results performed as above are the same as shown in a following table 6.

【Table 6】

Experimental group	Weight change g	Colon length cm	MPO activity μU/mg	TNF-α pg/mg	IL-17 pg/mg	NF-kB activity p-p65/p65	COX-2 activity
Normal group	0.9	6.5	0.21	35	17	0.12	0.23

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Induced group	-1.9	4.4	1.86	265	89	0.32	0.52
LP NK3	-0.9	5.2	1.12	89	45	0.23	0.35
BL NK49	-0.7	5.4	0.87	95	38	0.22	0.36
Mixture	-0.7	5.5	0.82	88	35	0.19	0.29
Positive control group	-1.0	5.1	1.26	105	47	0.25	0.42

Particularly, it was identified that there is no great change in weight of the group dosed with Lactobacillus plantarum NK3, Bifidobacterium longum NK49 or a mixture thereof, thereby showing no toxicity. Also, when inducing colitis, a length of colon was decreased. However, in case of the group dosed with lactic acid bacteria, it was identified that there occurs an effect of recovering a length of colon. Furthermore, in case of the group dosed with lactic acid bacteria, it was identified that an activity of myeloperoxidase, which is increased according to induced colitis, is decreased, an expression level of TNF-α and IL-17 cytokines is inhibited, and activities of NF-kB and COX-2 are inhibited.

From the results, it was identified that the novel lactic acid bacteria have an effect of preventing and treating colitis, without showing any toxicity.

Accession information of lactic acid bacteria

The present inventors deposited Lactobacillus plantarum NK3 for the purpose of patent to the Korean Culture Center of Microorganisms, a certified depository institution (address: Yulim Building, 45, Hongjenae 2ga-gil, Seodaemun-gu, Seoul, South Korea) on August 04, 2017, and received an accession number of KCCM12089P.

Also, the present inventors deposited Bifidobacterium longum NK49 for the purpose of patent to the Korean Culture Center of Microorganisms, a certified depository institution (address: Yulim Building, 45, Hongjenae 2ga-gil, Seodaemun-gu, Seoul, South Korea) on August 04, 2017, and received an accession number of KCCM12088P.

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Translation

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

INTERNATIONAL FORM

To. KIM DONG HYUN
26, Kyungheedae-ro,
Dongdaemun-gu,
Seoul, 02447,
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 : ***Bifidobacterium longum* NK49**	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **KCCM12088P**
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 **August. 04. 2017.** (date of the original deposit)[1]	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : **Korean Culture Center of Microorganism** Address: Yurim B/D 45, Hongjenae-2ga-gil Seodaemun-gu SEOUL 120-861 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: **August. 04. 2017.**

1 Were 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.

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Translation

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I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR : ***Lactobacillus plantarum NK3***	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: **KCCM12089P**
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 **August. 04. 2017.** (date of the original deposit)[1]	
IV. INTERNATIONAL DEPOSITARY AUTHORITY	
Name : **Korean Culture Center of Microorganism** Address: Yurim B/D 45, Hongjenae-2ga-gil Seodaemun-gu SEOUL 120-861 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): Date: **August. 04. 2017.**

1 Were 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.

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Claims

1. Isolated *Bifidobacterium longum NK49*, as deposited at the Korean Culture Center of Microorganisms under accession number KCCM12088P.

2. The isolated *Bifidobacterium longum NK49* KCCM12088P according to claim 1, wherein said isolated *Bifidobacterium longum NK49* KCCM12088P comprises a 16S rDNA sequence of SEQ ID NO: 2.

3. A pharmaceutical composition comprising the isolated *Bifidobacterium longum NK49* KCCM12088P of claim 1 or 2.

4. The pharmaceutical composition according to claim 3, wherein said isolated *Bifidobacterium longum NK49* KCCM12088P is a live bacterial cell thereof, a dead bacterial cell thereof, a culture product thereof, or a crushed product thereof.

5. A health functional food comprising the isolated *Bifidobacterium longum NK49* KCCM12088P of claim 1 or 2.

6. The health functional food according to claim 5, wherein said isolated *Bifidobacterium longum NK49* KCCM12088P is a live bacterial cell thereof, a dead bacterial cell thereof, a culture product thereof, or a crushed product thereof.

7. Use of the isolated *Bifidobacterium longum NK49* KCCM12088P of claim 1 or 2 in the manufacture of a medicament for the prevention or treatment of inflammatory diseases,

wherein said inflammatory disease is at least one selected from the group consisting of hepatitis, obesity, gastritis, enteritis, nephritis, colitis, diabetes, pancreatitis, urethritis, cystitis and vaginitis.

8. The use according to claim 7, wherein said inflammatory disease is colitis or vaginitis.

9. Use of the isolated *Bifidobacterium longum NK49* KCCM12088P of claim 1 or 2 for prevention or treatment of inflammatory diseases,

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wherein said inflammatory disease is at least one selected from the group consisting of hepatitis, obesity, gastritis, enteritis, nephritis, colitis, diabetes, pancreatitis, urethritis, cystitis and vaginitis.

10. The use according to claim 9, wherein said inflammatory disease is colitis or vaginitis.

11. A method for preventing or treating inflammatory diseases comprising administering to a subject in need thereof the isolated *Bifidobacterium longum NK49 KCCM12088P* of claim 1 or 2,

wherein said inflammatory disease is at least one selected from the group consisting of hepatitis, obesity, gastritis, enteritis, nephritis, colitis, diabetes, pancreatitis, urethritis, cystitis and vaginitis.

12. The method according to claim 11, wherein said inflammatory disease is colitis or vaginitis.

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FIG. 1

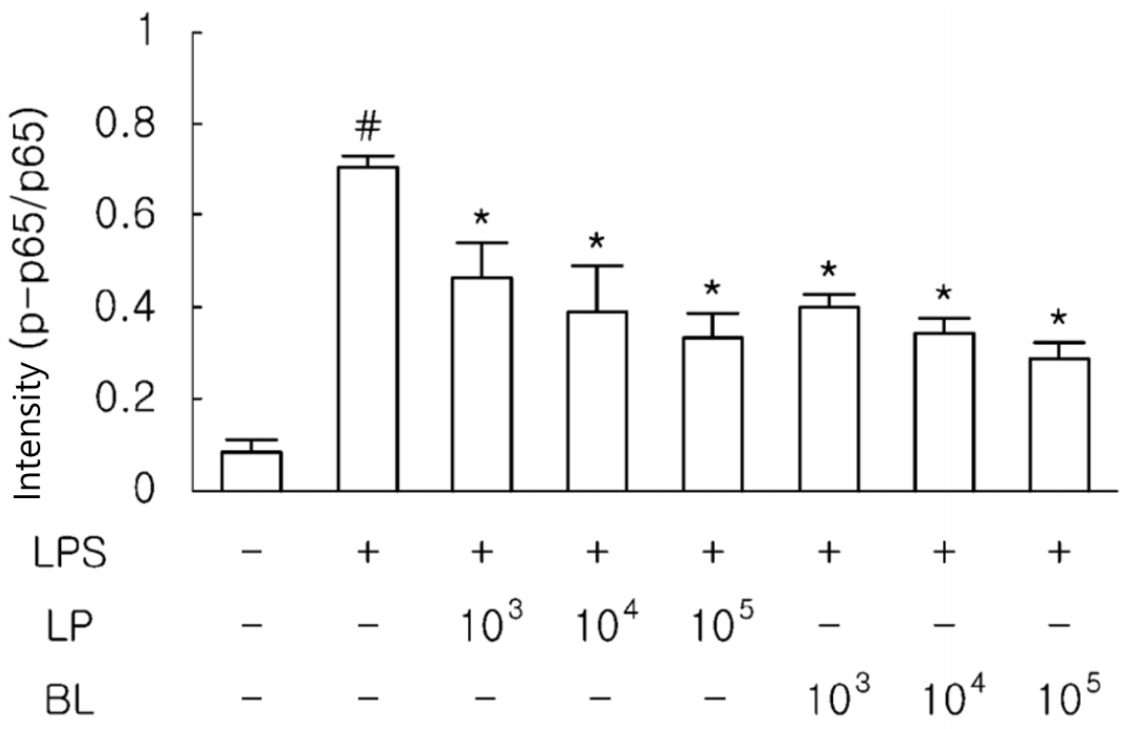
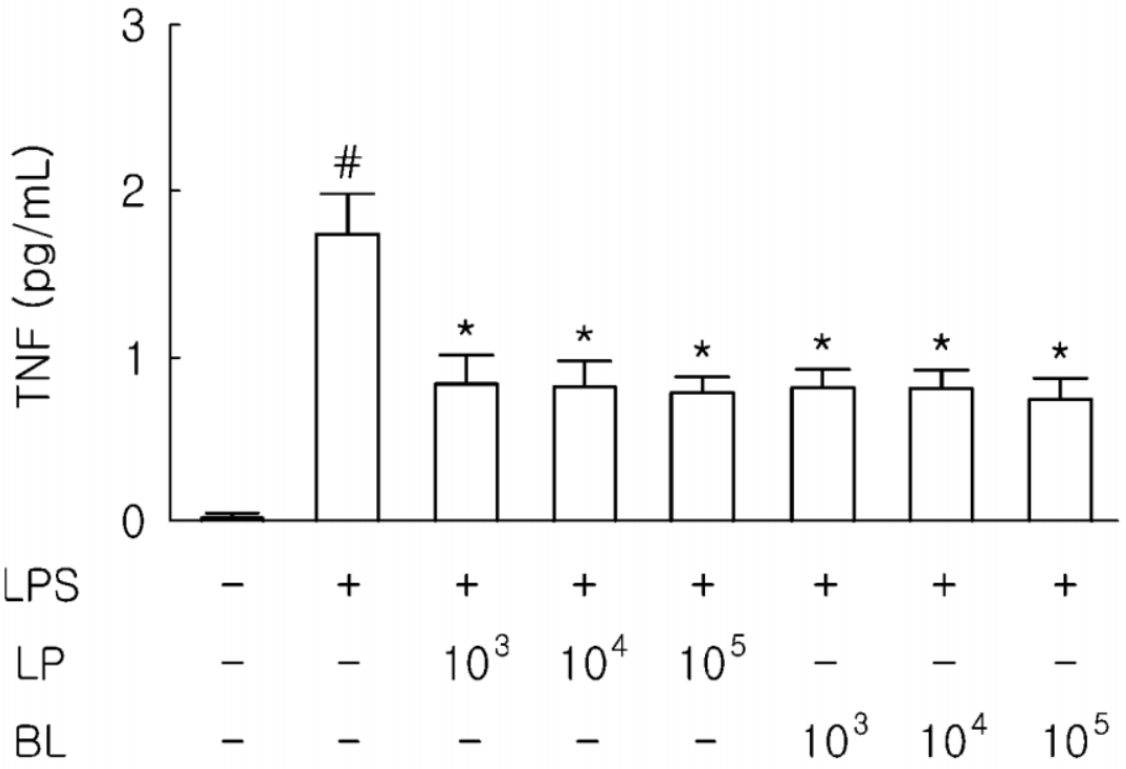


FIG. 2



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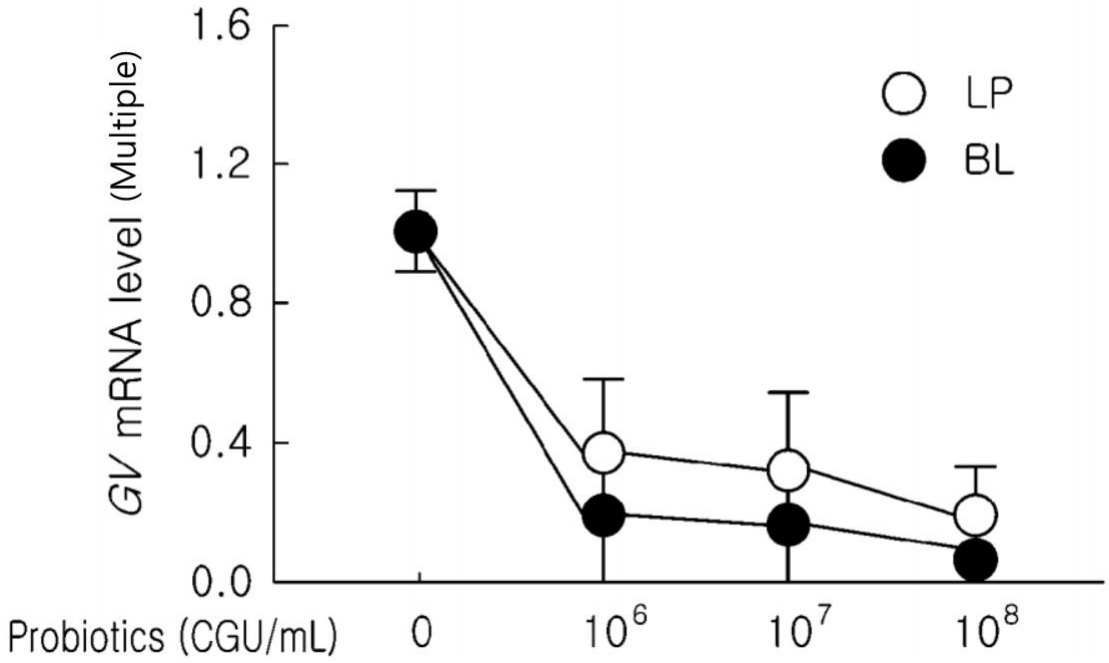

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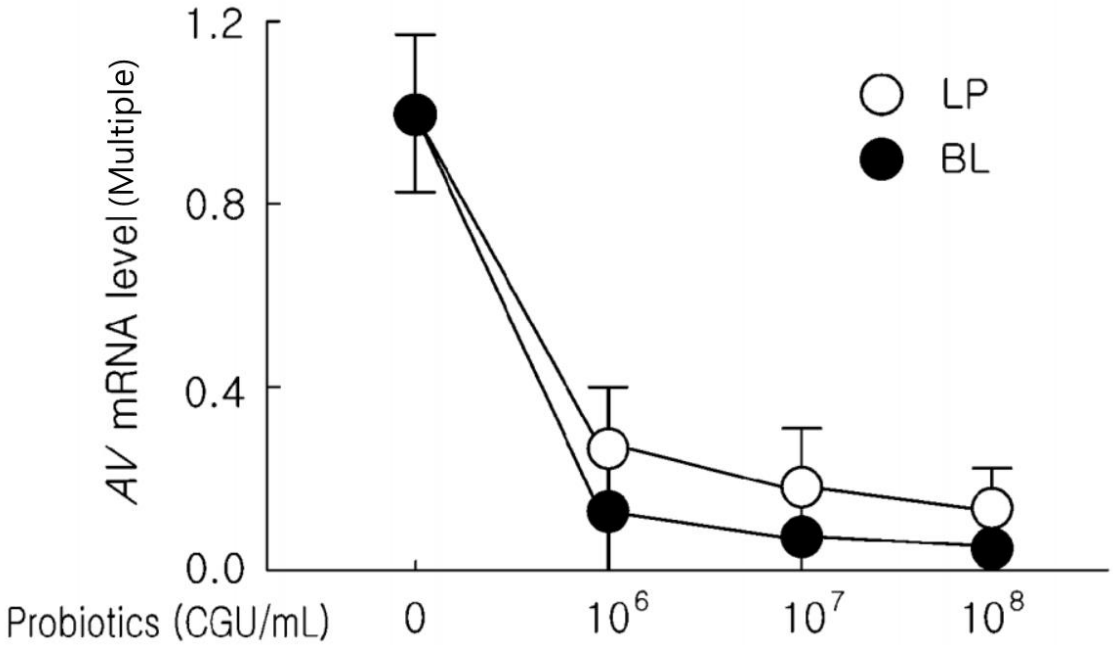

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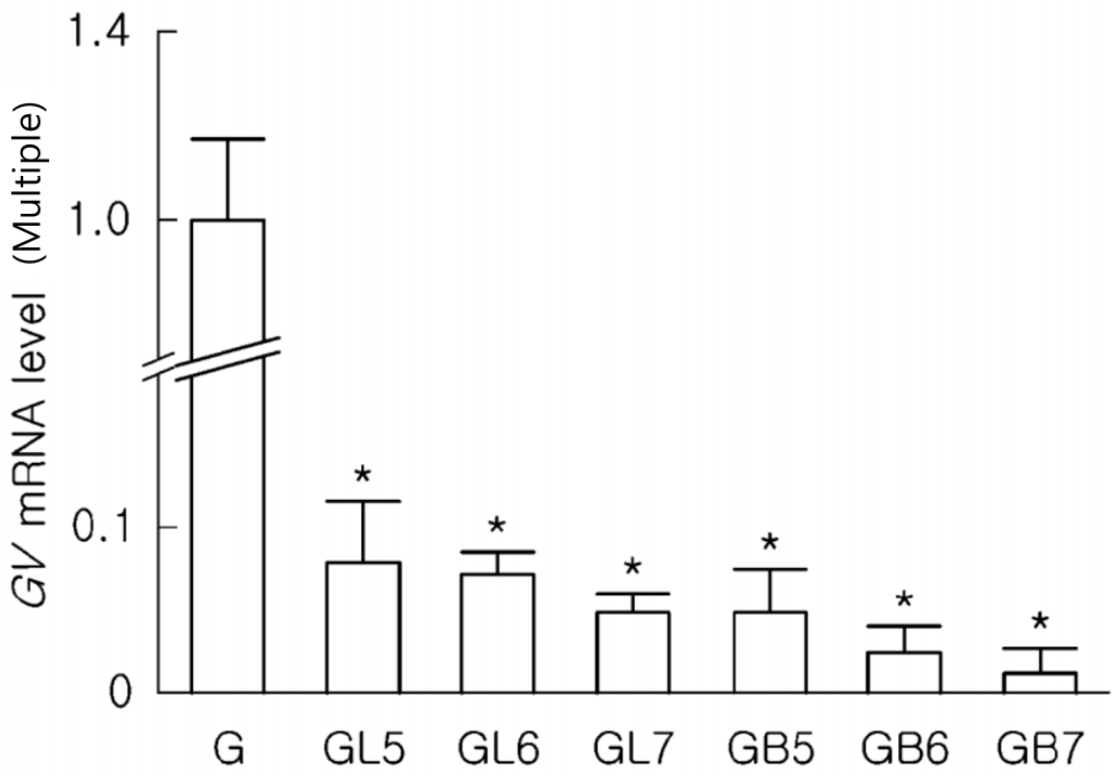

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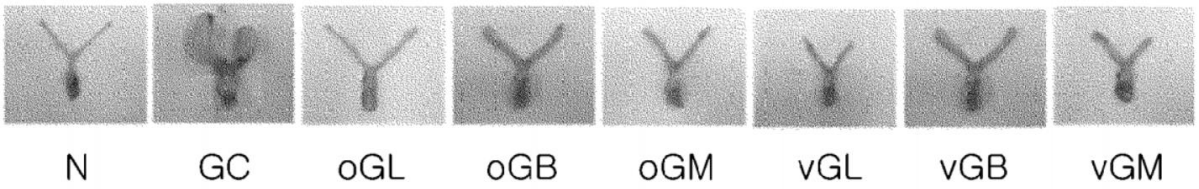

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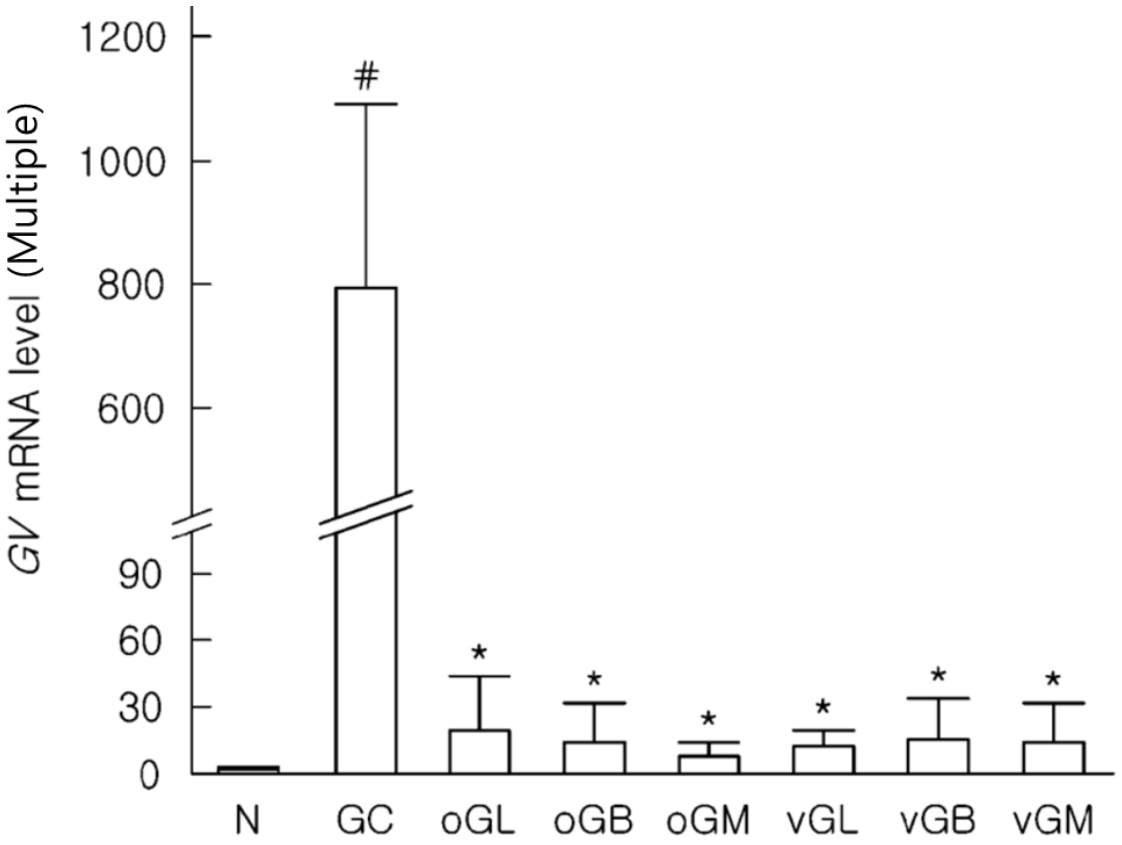

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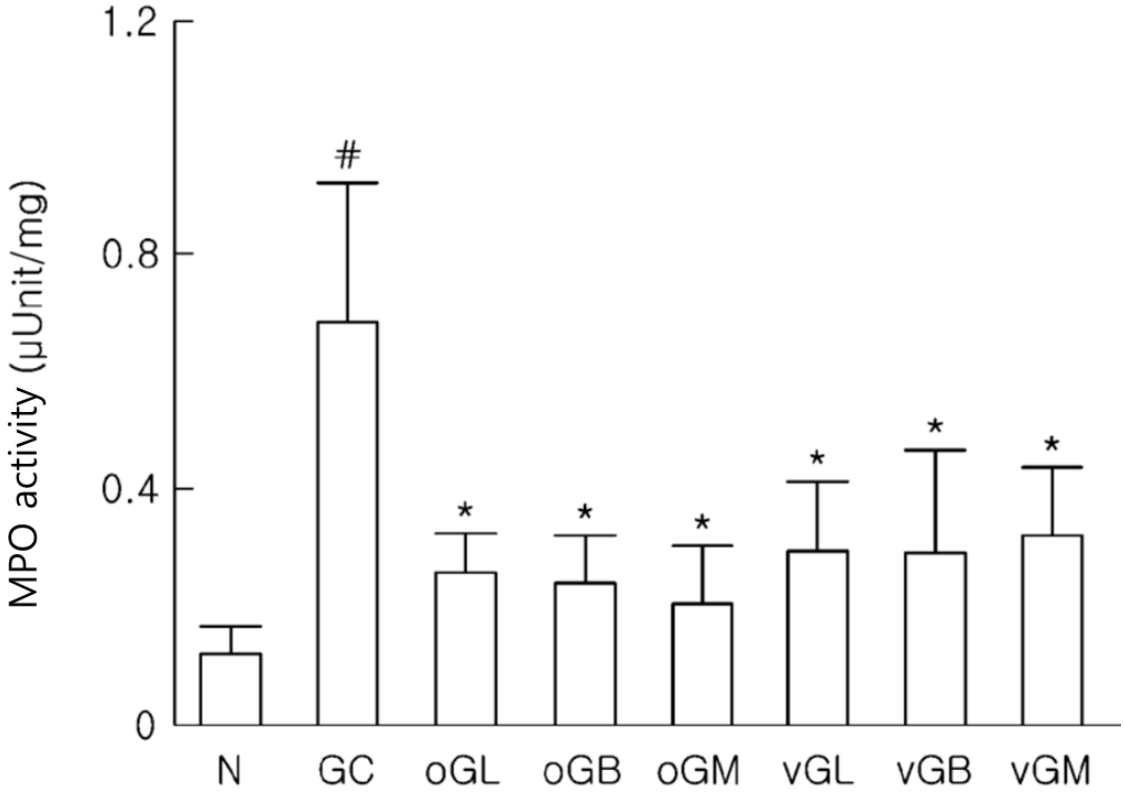

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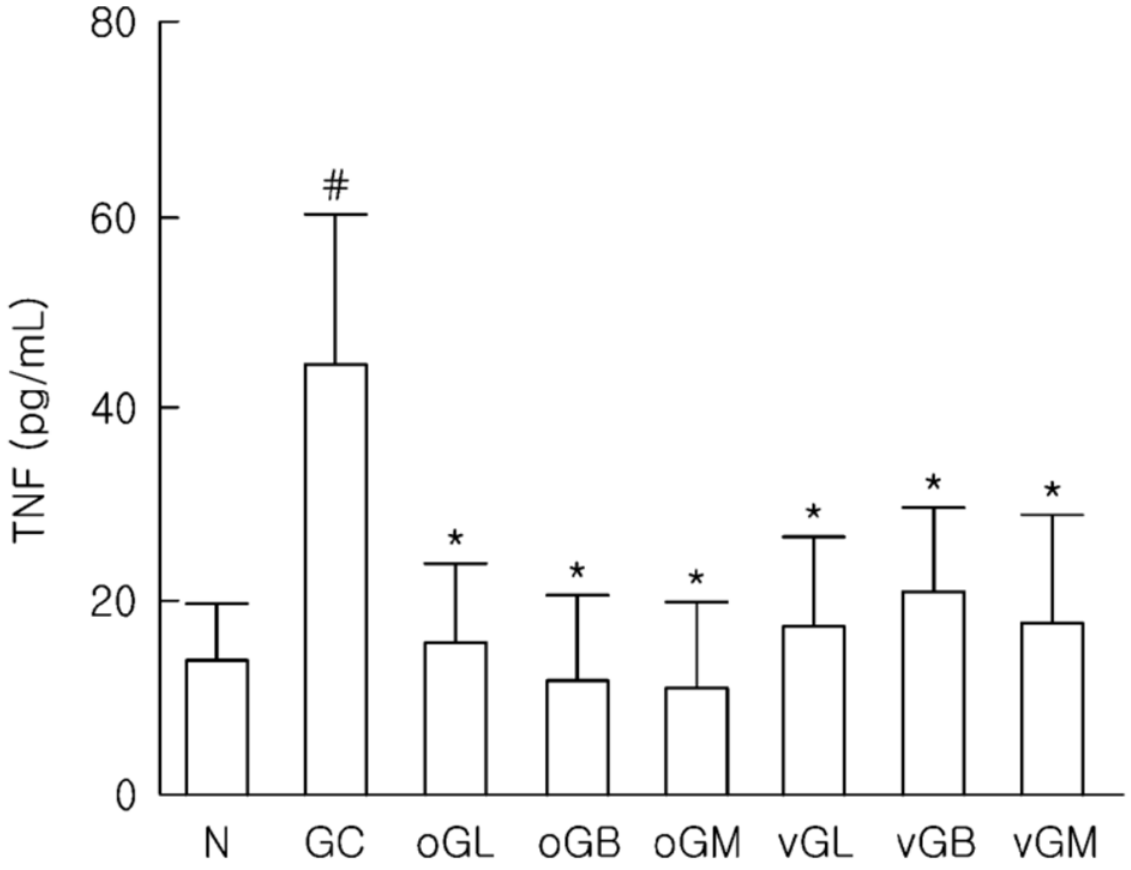

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FIG. 10

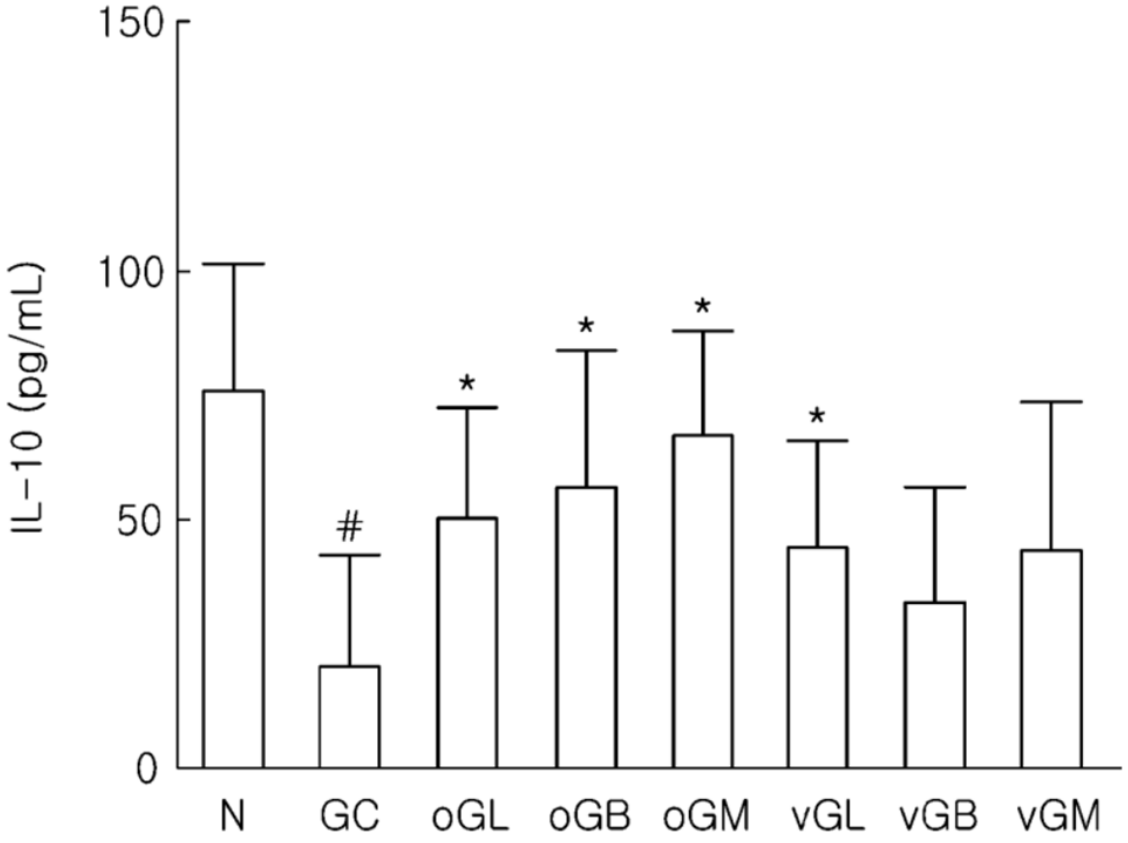
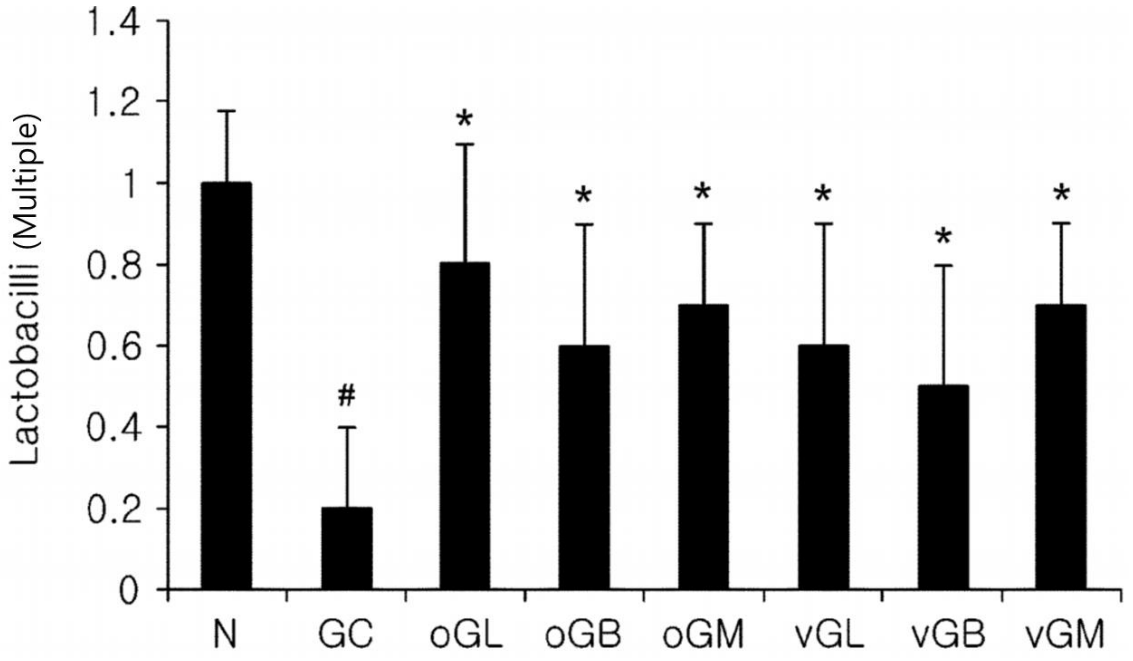


FIG. 11



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