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Novel lactobacillus and composition for preventing, improving, or treating degenerative brain diseases or cognitive function disorders

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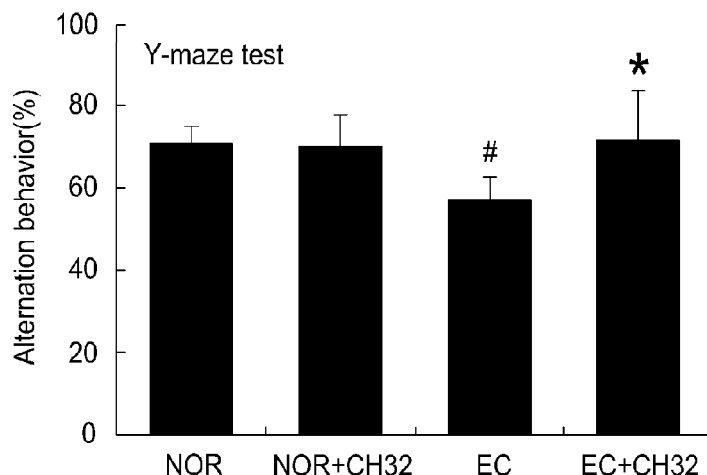
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공개:

- 국제조사보고서와 함께 (조약 제 21 조(3))
- 청구범위 보정 기한 만료 전의 공개이며, 보정서를 접수하는 경우 그에 관하여 별도 공개함 (규칙 48.2(h))
- 명세서의 서열목록 부분과 함께 (규칙 5.2(a))

(54) Title: NOVEL LACTOBACILLUS AND COMPOSITION FOR PREVENTING, IMPROVING, OR TREATING DEGENERATIVE BRAIN DISEASES OR COGNITIVE FUNCTION DISORDERS

(54) 발명의 명칭 : 신규 유산균 및 퇴행성 뇌질환 또는 인지기능 장애의 예방, 개선 또는 치료용 조성물



(57) Abstract: The present invention provides a novel lactobacillus and a use for same, the lactobacillus being separated from kimchi and thus highly safe, and having various types of biological activity such as memory improving activity, tight junction protein expression inducing activity, antioxidation activity, lipopolysaccharide (LPS) generation-suppressing activity, and β -glucuronidase inhibiting activity. The novel lactobacillus according to the present invention can be used as a functional food medicine material for preventing, improving, or treating degenerative brain diseases or cognitive function disorders.

(57) 요약서:

[다음 쪽 계속]

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본 발명은 김치에서 분리되어 안전성이 높고 기억력 개선 활성, 밀착연접단백질(tight junction protein) 발현 유도 활성, 항산화 활성, 지질다당류(lipopolysaccharide, LPS) 생성 억제 활성 또는 베타-글루쿠로니다제(β -glucuronidase) 저해 활성 등과 같은 다양한 생리활성을 갖는 신규 유산균 및 이의 용도를 제공한다. 본 발명에 따른 신규 유산균은 퇴행성 뇌 질환 또는 인지기능 장애의 예방, 개선 또는 치료를 위한 기능성 식의약 소재로 사용될 수 있다.

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【DESCRIPTION】**【Invention Title】**

NOVEL LACTOBACILLUS AND COMPOSITION FOR PREVENTING,
IMPROVING, OR TREATING DEGENERATIVE BRAIN DISEASES OR COGNITIVE
5 FUNCTION DISORDERS

【Technical Field】

The present invention relates to novel lactic acid
bacteria and the use thereof, and more particularly to novel
lactic acid bacteria, which is isolated from Kimchi, having
10 various types of physiological activities, such as memory
improvement activity, tight junction protein expression
inducement activity, antioxidant activity,
lipopolysaccharide(LPS) production-inhibitory activity or β -
glucuronidase-inhibitory activity, and to food and medicinal
15 uses of novel lactic acid bacteria for preventing, alleviating
or treating degenerative brain diseases or cognitive function
disorders.

【Background Art】

With the rise of aging society, the number of patients
20 with degenerative brain diseases, such as Alzheimer's disease,
Parkinson's disease and dementia is rapidly increasing.
Alzheimer's disease, the most common degenerative brain disease
that causes dementia, slowly develops and gradually
deteriorates cognitive functions including memory. While the
25 exact cause of Alzheimer's disease has yet to be found, it is
closely associated with aging and is growing with the increase

of the older population.

Parkinson's disease is a chronic progressive degenerative disease of the nerve system caused by the loss of dopamine neurons, showing symptoms of muscle cramps, bradykinesia (slowness of movement) and postural instability. Patients with Alzheimer's disease show symptoms of dementia, such as speech impairment and executive function inability while patients with Parkinson's disease show symptoms of dementia, such as lapses in concentration and visual/spatial judgment, executive function disorder and slowness in thinking.

Dementia may be defined as a condition where a physically stable person experiences damage to brain function due to various causes and gradual deterioration of the overall cognitive ability, greatly affecting daily life activities. Cognitive ability herein means various intellectual abilities, such as memory, speech, visual-spatial ability, judgment and abstract thinking. Each of the cognitive abilities is deeply associated with a certain part of brain. About 50% of dementia are a Alzheimer's disease-type, 20 to 30% of dementia are a blood vessel-type and there are other types of dementia, such as alcohol-related dementia, dementia with Lewy bodies, frontotemporal dementia, and Parkinson's disease dementia. Recently, there have been studies that various degenerative brain diseases including dementia are associated with Intestinal Permeability Syndrome or intestinal flora disturbance.

Intestinal Permeability Syndrome and Dementia

The gastrointestinal tract of the human body is composed of mucus and villi, which efficiently absorb nutrient components, but prevent the absorption of pathogenic microorganisms having a high molecular weight or toxins produced by these microorganisms. In addition, the human body has an immune system capable of protecting the body from invasion of external antigens having a high molecular weight. However, due to infection with many pathogenic microorganisms or toxins, excessive stress, intake of foods such as high-fat diets capable of proliferating harmful bacterial living in the gastrointestinal tract, excessive alcohol intake, the abuse of drugs (e.g., antibiotics) and the like, intestinal flora is disturbed, abnormalities in the gastrointestinal tract's immune system occur, and expression of tight junction proteins is inhibited. If expression of tight junction proteins is inhibited, tight junction of intestinal mucosa becomes loosened, and the invasion into the body of large molecules due to the loosened gap and abnormalities in the immune system. Intestinal permeability syndrome is also known as leaky gut syndrome, and refers to a condition in which external such as less digested foods, pathogenic microorganisms, toxins or the like are continuously introduced into blood, because the tight junction barrier system of epithelial cells forming the gastrointestinal tract is not smoothly operated. When intestinal permeability syndrome occurs, external antigens that are generally not absorbed into the body enter the body, thus

causing ulcerative colitis, Crohn's disease, liver injury, liver dysfunction, allergic diseases (including asthma), atopy, autoimmune diseases, steatorrhea, digestive absorption disorder, acne, accelerated aging, endotoxemia, intestinal infection, 5 eczema, irritable bowel syndrome, chronic fatigue syndrome, psoriasis, rheumatoid arthritis, pancreatic insufficiency, inflammatory joint diseases or the like. Recently, there have been studies that Intestinal Permeability Syndrome is associated with dementia caused by Parkinson's disease or aging.

10 Intestinal Flora Disturbance and Dementia

There are many bacteria living in the gastrointestinal tract of the human body. The human body has about 10 trillion normal cells, but has about 100 trillion bacteria which are about 10-fold larger than the normal cells. These bacteria may 15 be divided into beneficial bacteria that help human intestinal health and harmful bacteria that are harmful to human health. The health of human body may be maintained when beneficial bacterial such as *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Sporolactobacillus* and the like are 20 more dominant in the gastrointestinal tract than harmful bacteria. Otherwise, diseases may be caused, such as obesity, intestinal permeability syndrome, liver diseases, accelerated aging, enteritis, accelerated aging, dementia and the like. Increase of harmful microorganisms in the intestinal flora (ex. 25 *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis*, etc.) boosts the activity of NF- κ B in intestinal cells, which can dramatically increase the possibility of degenerative brain

diseases, such as Alzheimer's disease and Parkinson's disease, and dementia.

Probiotics are collectively referred to as live microorganisms that improve the host's microbial environment in the gastrointestinal tract of animals, including humans, and have beneficial effects on the host's health. In order to be effective as probiotics, it is necessary to have excellent acid resistance, bile resistance and adherence to epithelial cells, because most of these probiotics should reach the small intestine upon oral administration and must be adhered to the intestinal surface. Lactic acid bacteria are used as probiotics because they play a role in decomposing fibrous and complex proteins to make important nutrients while living in the digestive system of the human body. Lactic acid bacteria have been reported to exhibit effects such as maintenance of intestinal normal flora, improvement of intestinal flora, anti-diabetic and anti-hyperlipidemic effects, inhibition of carcinogenesis, inhibition of colitis, and nonspecific activity of the host's immune system. Among these lactic acid bacteria, *Lactobacillus* sp. strains are major members of normal microbial communities living in the bowel of the human body and have long been known to be important in maintaining a healthy digestive tract and vaginal environment. Currently, according to the U.S. Public Health Service guidelines, all the *Lactobacillus* strains deposited with the American Type Culture Collection (ATCC) are classified as 'Bio-Safety Level 1', which is recognized as having no known potential risk of causing disease in humans or

animals. Meanwhile, lactic acid bacteria of kimchi that are involved in kimchi fermentation have been reported to have immune enhancement effects, antimicrobial effects, antioxidant effects, anti-cancer effects, anti-obesity effects, hypertension preventive effects or constipation preventive effects [Hivak P, Odrska J, Ferencik M, Ebringer L, Jahnova E, Mikes Z. : One-year application of Probiotic strain *Enterococcus faecium* M-74 decreases Serum cholesterol levels. : Bratisl lek Listy 2005; 106(2); 67-72; Agerholm-Larsen L. Bell ML. Grunwald GK. Astrup A. : The effect of a probiotic milk product on plasma cholesterol : a metaanalysis of short-term intervention studies ; Eur J Clin Nutr. 2000; 54(11) 856-860; Renato Sousa, Jaroslava Helper, Jian Zhang, Strephen J Lewis and Wani O Li ; Effect of *Lactobacillus acidophilus* supernants on body weight and leptin expression in rats; BMC complementary and alternative medicine. 2008; 8(5)1-8].

Since various bioactive activities of lactic acid bacteria were known, There have recently been a growing number of studies designed to develop a safe and highly functional lactic acid bacteria flora for the human body and turn it into an ingredient of medicinal products or functional foods. For example, the Korean Patent Gazette for registration No. 10-1476236 discloses a pharmaceutical composition for prevention or treatment of dementia, comprising *lactobacillus pentosus* var. *plantarum* C29 KCCM11291P flora as an active ingredient. The Korean Patent Gazette for registration No. 10-1087972 discloses the preparation method of lactic acid bacteria ferment that is

effective in preventing and treating dementia, comprising (a) a stage to inoculate, culture and ferment lactic acid bacteria selected from *Lactobacillus* sp., *Enterococcus* sp. and *Bifidobacterium* sp. into the medium comprising milk; (b) a
5 stage to remove lactic acid bacteria from the above ferment; and (c) a stage to precipitate separate active ingredients adding a solvent selected from a group consisting of acetone and alcohol having 1 to 6 carbon atoms to the ferment, which had lactic acid bacteria removed. In addition, the Korean
10 Patent Gazette for registration No. 10-1424547 discloses a pharmaceutical composition for preventing or treating degenerative brain diseases comprising the lactic acid bacteria ferment of *Lactobacillus fermentum* KFRI 164 of Sibjeondaebotang, as an active ingredient. Also, the Korean Patent Gazette for
15 publication No. 10-2015-0047687 discloses a composition for preventing or improving forgetfulness or improving memory, comprising the plant extract that was fermented by adding 0.1 to 10 wt% of glucose and 0.1 to 5 wt% of yeast extract to a plant extract, hot-water extracted from *Polygala tenuifolia*,
20 white *Poria cocos* Wolf, and *Acoris gramineus*, and then inoculating the cultured *Lactobacillus plantarum*.

And yet, lactic acid bacteria disclosed in the prior art or the product fermented by the same is not enough to be applied as a commercial treatment since it is not highly
25 effective in treating degenerative brain diseases including dementia. As such, it is necessary to screening a certain type of lactic acid bacteria that has the equivalent level of

treatment effect for brain diseases compared to commercial treatments, and improves Intestinal Flora Disturbance and Intestinal Permeability Syndrome, thereby developing medicinal products or functional foods.

5 Reference to any prior art in the specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in Australia or any other jurisdiction or that this prior art could reasonably be expected to be ascertained,
10 understood and regarded as relevant by a person skilled in the art.

【Disclosure】

【Technical Problem】

The present invention has been made under the Background
15 Art as described above, and it is an aspect of the present invention to provide novel lactic acid bacteria having various physiological activities or functionalities required for probiotics.

In addition, an aspect of the present invention is to
20 provide a composition that comprises novel lactic acid bacteria or ferment thereof and may be used to alleviate, prevent or treat degenerative brain diseases.

【Technical Solution】

To achieve the above aspect, an embodiment of the present
25 invention is *Lactobacillus johnsonii* comprising 16S rDNA nucleotide sequence represented by SEQ ID NO: 2, which provides

the lactic acid bacteria having the memory improvement activity, tight junction protein expression inducement activity, antioxidant activity, lipopolysaccharide (LPS) production inhibitory activity or β -glucuronidase inhibitory activity.

5 To achieve the above aspect, an embodiment of the present invention provides a pharmaceutical composition for preventing or treating degenerative brain diseases or cognitive function disorder comprising a lactic acid bacteria corresponding to *Lactobacillus johnsonii* comprising a 16S rDNA nucleotide
10 sequence represented by SEQ ID NO: 2, a culture of the lactic acid bacteria, a lysate of the lactic acid bacteria or an extract of the lactic acid bacteria as an active ingredient. Still another embodiment of the present invention provides a food composition for preventing or alleviating degenerative
15 brain diseases or cognitive function disorder comprising a lactic acid bacteria corresponding to *Lactobacillus johnsonii* comprising a 16S rDNA nucleotide sequence represented by SEQ ID NO: 2, a culture of the lactic acid bacteria, a lysate of the lactic acid bacteria or an extract of the lactic acid bacteria
20 as an active ingredient. Still another embodiment of the present invention provides a food composition for improving memory or learning ability comprising a lactic acid bacteria corresponding to *Lactobacillus johnsonii* comprising a 16S rDNA nucleotide sequence represented by SEQ ID NO: 2, a culture of
25 the lactic acid bacteria, a lysate of the lactic acid bacteria or an extract of the lactic acid bacteria as an active ingredient.

To achieve the above aspect, another embodiment of the present invention provides a pharmaceutical composition for preventing or treating degenerative brain diseases or cognitive disorder, comprising red bean ferment or extract of the red bean ferment as an active ingredient. Also, another embodiment of the present invention provides a food composition for preventing or alleviating degenerative brain diseases or cognitive function disorder, comprising red bean ferment or extract from the red bean ferment. In addition, another embodiment of the present invention provides a food composition for improving memory or learning ability, comprising red bean ferment or extract from the red bean ferment. In the composition according to another embodiment of the present invention, the above red bean ferment is a product that was fermented with lactic acid bacteria that comprises 16S rDNA nucleotide sequence represented by SEQ ID NO: 2 and corresponds to *Lactobacillus johnsonii*.

【Advantageous Effects】

A certain *Lactobacillus* sp. strain according to the present invention is isolated from kimchi, and thus is highly safe, and has various physiological activities such as memory improvement activity, tight junction protein expression inducement activity, antioxidant activity, lipopolysaccharide (LPS) production inhibitory activity or β -glucuronidase inhibitory activity. Accordingly, a certain *Lactobacillus* sp. strain according to the present invention may be used as an ingredient of medicinal products or functional foods for

preventing, alleviating or treating various diseases through combination of the strain's first effect that alleviates intestinal permeability and the secondary effect that improves learning ability or memory.

5 **【Description of Drawings】**

FIG. 1 is a graph showing the impact that administration of *Lactobacillus johnsonii* CJLJ103 has on the Y-maze test of model animals induced to have memory impairment by *Escherichia coli* K20.

10 FIG. 2 is a graph showing the impact that administration of *Lactobacillus johnsonii* CJLJ103 has on the Passive Avoidance test of model animals induced to have memory impairment by *Escherichia coli* K20.

FIG.3 is a photo showing the impact that administration
15 of *Lactobacillus johnsonii* CJLJ103 has on the expression level of nerve growth-promoting factor of model animals induced to have memory impairment by *Escherichia coli* K20.

【Mode for Invention】

As used herein, terms used in the present invention will
20 be defined.

As used herein, the term "cognitive function disorder" means a symptom or disease where cognitive functions, such as memory-processing, cognition or problem-solving do not fully function, specifically including declining working memory,
25 attention and vigilance, linguistic learning and memory, visual

learning and memory, and reasoning and problem-solving, namely, executive function, processing speed or social cognition.

As used herein, the term "brain disease" means all types of diseases generated in the brain due to destroyed cranial
5 nerve cells.

As used herein, the term "culture" means a product obtained by culturing a microorganism in a known liquid medium or solid medium, and thus is intended to include a microorganism.

10 As used herein, the term "ferment" means a product yielded by fermenting the raw ingredient, a subject of fermentation, with microorganism, and a concept comprising microorganism.

As used herein, the term "lactic acid bacteria, etc." 15 means lactic acid bacteria, and culture, lysate or extract of the lactic acid bacteria.

As used herein, the term "red bean ferment, etc." means the red bean ferment or extract of the ferment.

As used herein, the terms "pharmaceutically acceptable" 20 and "sitologically acceptable" means neither significantly stimulating an organism nor inhibiting the biological activity and characteristics of an active material administered.

As used herein, the term "preventing" refers to all actions that inhibit symptoms or delay the progression of a
25 particular disease by administrating the composition of the present invention.

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As used herein, the term "treating" refers to all actions that alleviate or beneficially change the symptoms of a particular disease by administering the composition of the present invention.

5 As used herein, the term "alleviating" refers to all actions that at least reduce a parameter related to the condition to be treated, for example, the degree of symptom.

As used herein, the term "administering" means providing the composition of the present invention to a subject by any
10 suitable method. As used herein, the term "subject" means all animals, including humans, monkeys, dogs, goats, pigs or rats, which have a particular disease whose symptoms may be alleviated by administering the composition of the present invention.

15 As used herein, the term "pharmaceutically effective amount" refers to an amount sufficient to treat diseases, at a reasonable benefit/risk ratio applicable to any medical treatment. The pharmaceutically effective amount may be determined depending on factors including the kind of subject's
20 disease, the severity of the disease, the activity of the drug, sensitivity to the drug, the time of administration, the route of administration, excretion rate, the duration of treatment and drugs used in combination with the composition, and other factors known in the medical field.

25 Hereinafter, the present invention will be described in detail.

One aspect of the present invention is related to novel lactic acid bacteria having various physiological activities.

A lactic acid bacteria according to one embodiment of the present invention is *Lactobacillus johnsonii* comprising 16S
5 rDNA nucleotide sequence represented by SEQ ID NO: 2, having memory improvement activity, tight junction protein expression inducement activity, antioxidant activity, lipopolysaccharide (LPS) production inhibitory activity or β -glucuronidase inhibitory activity. The *Lactobacillus johnsonii* is an
10 anaerobic bacillus isolated from kimchi, is positive to gram staining, can survive in a wide temperature range and low pHs, and produces glucosidase. Furthermore, the *Lactobacillus johnsonii* utilizes D-glucose, D-fructose, D-mannose, N-acetyl-glucosamine, maltose, lactose, sucrose, gentibiose and the like
15 as carbon sources. In addition, the *Lactobacillus johnsonii* is preferably *Lactobacillus johnsonii* CJLJ103 (accession number: KCCM 11763P).

One aspect of the present invention relates to a composition comprising a particular lactic acid bacteria, etc.,
20 as an active ingredient. The composition according to one embodiment of the present invention comprises 16S rDNA nucleotide sequence represented by SEQ ID NO: 2, and also comprises lactic acid bacteria corresponding to *Lactobacillus johnsonii*, culture, lysate or extract thereof as an active
25 ingredient. The *Lactobacillus johnsonii*, is an anaerobic bacillus isolated from kimchi and positive to gram staining, and survives in a wide range of temperatures with low PH

environment and produces glucosidase. Also, the *Lactobacillus johnsonii* uses D-glucose, D-fructose, D-mannose, N-acetyl-glucosamine, Maltose, Lactose, Sucrose, Gentiobiose and the like as a carbon source. In addition, the *Lactobacillus*
5 *johnsonii* is preferably *Lactobacillus johnsonii* CJLJ103 (accession number: KCCM 11763P).

In the present invention, a culture of the lactic acid bacteria is produced by culturing a certain strain or a mixture of strains in a medium. The medium may be selected
10 from known liquid media or solid media, and may be, for example, MRS liquid medium, MRS agar medium or BL agar medium.

The composition according to one embodiment of the present invention may be used to prevent, alleviate or treat degenerative brain diseases or cognitive function disorder,
15 since lactic acid bacteria, etc., the active ingredient, has various types of physiological activities, such as memory improvement activity, tight junction protein expression inducement activity, antioxidant activity, lipopolysaccharide (LPS) production inhibitory activity or β -glucuronidase
20 inhibitory activity. The degenerative brain disease may be Alzheimer's disease, Parkinson's disease, Huntington's disease, or dementia, specifically. Also, the dementia may be selected from a group consists of senile dementia, vascular dementia, Lewy body dementia, frontotemporal dementia, Alzheimer's
25 disease-type dementia, Parkinson's disease-type dementia, Huntington's disease-type dementia, Creutzfeldt-Jacob disease-type dementia, Pick's disease-type dementia, normal pressure

hydrocephalus-causing dementia and head injury-causing dementia. Also, the composition according to one embodiment of the present invention may be used to improve memory or learning ability.

5 Another aspect of the present invention relates to a composition comprising a product fermented by lactic acid bacteria, etc., as an active ingredient. The composition according to another embodiment of the present invention comprises red bean ferment or extract of the same. In the
10 composition according to another embodiment of the present invention, the red bean ferment is a product that the red bean was fermented with lactic acid bacteria corresponding to *Lactobacillus johnsonii* and comprising 16S rDNA nucleotide sequence represented by SEQ ID NO: 2. In the composition
15 according to another embodiment of the present invention, the technical characteristics of the *Lactobacillus johnsonii* are as described above, and thus the description thereof is omitted.

Red bean ferment, an active ingredient of the composition according to another embodiment of the present invention, has
20 higher physiological activity than lactic acid bacteria and is more effective in the aspect of functional food and medicinal ingredient, since the ferment comprises both the secondary metabolite produced by the red bean fermentation and lactic acid bacteria used in the red bean fermentation. The
25 composition according to another embodiment of the present invention may be used to prevent, alleviate or treat degenerative brain diseases or cognitive function disorder. The

degenerative brain diseases may be Alzheimer's disease, Parkinson's disease, Huntington's disease, or dementia, specifically. Also, the dementias may be selected from a group consists of senile dementia, vascular dementia, Lewy body
5 dementia, frontotemporal dementia, Alzheimer's disease-type dementia, Parkinson's disease-type dementia, Huntington's disease-type dementia, Creutzfeldt-Jacob disease-type dementia, Pick's disease-type dementia, normal pressure hydrocephalus-causing dementia and head injury-causing dementia. Also, the
10 composition according to another embodiment of the present invention may be used to improve memory or learning ability.

In the present invention, the composition may be embodied as a pharmaceutical composition, a food additive, a food composition (particularly, a functional food composition), a
15 feed additive or the like depending on the intended use or aspect. In addition, the content of the lactic acid bacteria, etc. or red bean ferment, etc., as an active ingredient may also be adjusted within a wide range depending on the specific type, intended use or aspect of the composition.

20 The content of the lactic acid bacteria, etc. or red bean ferment, etc., as an active ingredient in the pharmaceutical composition according to the present invention is not particularly limited. For example, the content may be 0.01 to 99 wt%, preferably 0.5 to 50 wt%, more preferably 1 to 30 wt%,
25 based on the total weight of the composition. In addition, the pharmaceutical composition according to the present invention may further contain, in addition to the active ingredient,

additives such as pharmaceutically acceptable carriers, excipients or diluents. Carriers, excipients and diluents, which may be contained in the pharmaceutical composition according to the present invention, include lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starch, acacia gum, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methyl cellulose, microcrystalline cellulose, polyvinyl pyrrolidone, water, methyl hydroxybenzoate, propyl hydroxylbenzoate, talc, magnesium stearate and mineral oil. In addition, the pharmaceutical composition according to the present invention may further contain, in addition to the lactic acid bacteria, etc. or red bean ferment, etc., one or more active ingredients having the effect of preventing or treating degenerative brain diseases or cognitive function disorder. The pharmaceutical composition according to the present invention may be prepared as formulations for oral administration or formulations for parenteral administration, and the formulations may be prepared using diluents or excipients, such as fillers, extenders, binders, wetting agents, disintegrants, surfactants and the like, which are commonly used. Solid formulations for oral administration include tablets, pellets, powders, granules, capsules and the like, and such solid formulations may be prepared by mixing the active ingredient with at least one excipient, for example, starch, calcium carbonate, sucrose, lactose or gelatin. In addition to simple excipients, lubricants such as magnesium stearate or talc may also be used.

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Liquid formulations for oral administration include suspensions, solutions, emulsions and syrup, and may contain various excipients, for example, wetting agents, flavoring agents, aromatics, preservatives and the like, in addition to
5 water and liquid paraffin which are frequently used simple diluents. Formulations for parenteral administration include sterilized aqueous solutions, non-aqueous solutions, suspensions, emulsions, freeze-dried preparations and suppositories. Propylene glycol, polyethylene glycol, plant
10 oils such as olive oil, injectable esters such as ethyl oleate and the like may be used as non-aqueous solvents or suspending agents. As the base of the suppositories, witepsol, Macrogol, Tween 61, cacao butter, laurin fat, glycerogelatin and the like may be used. Furthermore, the composition may preferably be
15 formulated depending on each disease or component by a suitable method known in the art or the method disclosed in Remington's Pharmaceutical Science (the latest edition), Mack Publishing Company, Easton PA. The pharmaceutical composition of the present invention may be administered orally or parenterally to
20 mammals, including humans, according to a desired method. Routes for parenteral administration include skin external application, intraperitoneal injection, intrarectal injection, subcutaneous injection, intravenous injection, intramuscular injection, intrathoracic injection or the like. The dose of
25 the pharmaceutical composition of the present invention is not particularly limited as long as it is a pharmaceutically effective amount. The dose may vary depending on the patient's

weight, age, sex, health condition, diet, administration time, administration mode, excretion rate and the severity of the disease. The daily dose of the pharmaceutical composition of the present invention is not particularly limited, but is
5 preferably 0.1 to 3000 mg/kg based on an active ingredient, more preferably 1 to 2000 mg/kg based on an active ingredient and may be administered once or several times a day.

Furthermore, the content of the lactic acid bacteria, etc. or red bean ferment, etc., as an active ingredient in the food
10 composition according to the present invention is 0.01 to 99 wt%, preferably 0.1 to 50 wt%, more preferably 0.5 to 25 wt%, based on the total weight of the composition, but is not limited thereto. The food composition of the present invention may be in the form of pellets, powders, granules, infusions,
15 tablets, capsules, liquid or the like, and specific examples of the food may include meats, sausages, breads, chocolates, candies, snacks, confectionaries, pizzas, ramens, other noodles, gums, dairy products including ice creams, various kinds of soups, beverages, teas, functional water, drinks,
20 alcoholic beverages, vitamin complexes and the like, and may include all health foods in a general sense. The food composition of the present invention may further contain sitologically acceptable carriers, various flavoring agents or natural carbohydrates as additional ingredients, in addition to
25 the active ingredient. Additionally, the food composition of the present invention may contain various nutrients, vitamins, electrolytes, flavoring agents, coloring agents, pectic acid

and its salt, alginic acid and its salt, an organic acid, a protective colloidal thickener, a pH adjusting agent, a stabilizer, a preservative, glycerin, alcohol, a carbonating agent used for carbonated drinks and the like. Additionally, 5 the food composition of the present invention may contain fruit flesh for preparing natural fruit juices, fruit juice drinks and vegetable drinks. These ingredients may be used independently or as a mixture. The above-described natural carbohydrates may include monosaccharides such as glucose and 10 fructose, disaccharides such as maltose and sucrose, polysaccharides such as dextrin and cyclodextrin and sugar alcohols such as xylitol, sorbitol, and erythritol. As a flavoring agent, a natural flavoring agent such as thaumatin or a stevia extract, or a synthetic flavoring agent such as 15 saccharin or aspartame may be used.

Hereinafter, the present invention will be described in further detail with reference to examples. It is to be understood, however, that these examples are merely intended to clearly illustrate the technical characteristics of the present 20 invention and do not limit the scope of the present invention.

1. Isolation and Identification of Lactic Acid Bacteria

(1) Isolation of Lactic Acid Bacteria from Kimchi

Each of Chinese cabbage kimchi, radish kimchi and green 25 onion kimchi was crushed, and the crushed liquid was suspended in MRS liquid medium (MRS Broth; Difco, USA). Next, the supernatant was collected, transferred to MRS agar medium

(Difco, USA) and cultured anaerobically at 37°C for about 48 hours, and then strains that formed colonies were isolated.

(2) Isolation of Lactic Acid Bacteria from Human Feces

Human feces were suspended in GAM liquid medium (GAM
5 broth; Nissui Pharmaceutical, Japan). Next, the supernatant was collected, transferred to BL agar medium (Nissui Pharmaceutical, Japan) and cultured anaerobically at 37°C for about 48 hours, and then *Bifidobacterium* sp. strains that formed colonies were isolated.

10 (3) Identification of Screened Lactic Acid Bacteria

The physiological characteristics and 16S rDNA sequences of the strains isolated from kimchi or human feces were analyzed to identify the species of the strains, and names were given to the strains. Table 1 below the control numbers and
15 strain names of the lactic acid bacteria isolated from Chinese cabbage kimchi, radish kimchi, green onion kimchi and human feces.

Table 1

Control No.	Strain name	Control No.	Strain name
1	<i>Lactobacillus acidophilus</i> CH1	31	<i>Lactobacillus sakei</i> CH31
2	<i>Lactobacillus acidophilus</i> CH2	32	<i>Lactobacillus johnsonii</i> CJLJ103
3	<i>Lactobacillus acidophilus</i> CH3	33	<i>Lactobacillus sakei</i> CH33
4	<i>Lactobacillus brevis</i> CH4	34	<i>Lactobacillus sakei</i> CH34
5	<i>Lactobacillus curvatus</i> CH5	35	<i>Lactobacillus plantarum</i> CH35
6	<i>Lactobacillus brevis</i> CH6	36	<i>Lactobacillus</i> <i>sanfranciscensis</i> CH36
7	<i>Lactobacillus casei</i> CH7	37	<i>Bifidobacterium</i>

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			<i>pseudocatenulatum</i> CH37
8	<i>Lactobacillus planantrum</i> CH8	38	<i>Bifidobacterium</i> <i>pseudocatenulatum</i> CH38
9	<i>Lactobacillus sakei</i> CH9	39	<i>Bifidobacterium</i> <i>adolescentis</i> CH39
10	<i>Lactobacillus curvatus</i> CH10	40	<i>Bifidobacterium</i> <i>adolescentis</i> CH40
11	<i>Lactobacillus sakei</i> CH11	41	<i>Bifidobacterium</i> <i>adolescentis</i> CH41
12	<i>Lactobacillus curvatus</i> CH12	42	<i>Bifidobacterium animalis</i> CH42
13	<i>Lactobacillus plantarum</i> CH13	43	<i>Bifidobacterium animalis</i> CH43
14	<i>Lactobacillus fermentum</i> CH14	44	<i>Bifidobacterium bifidum</i> CH44
15	<i>Lactobacillus fermentum</i> CH15	45	<i>Bifidobacterium bifidum</i> CH45
16	<i>Lactobacillus gasseri</i> CH16	46	<i>Bifidobacterium breve</i> CH46
17	<i>Lactobacillus paracasei</i> CH17	47	<i>Bifidobacterium breve</i> CH47
18	<i>Lactobacillus helveticus</i> CH18	48	<i>Bifidobacterium breve</i> CH48
19	<i>Lactobacillus helveticus</i> CH19	49	<i>Bifidobacterium catenulatum</i> CH49
20	<i>Lactobacillus johnsonii</i> CH20	50	<i>Bifidobacterium catenulatum</i> CH50
21	<i>Lactobacillus johnsonii</i> CH21	51	<i>Bifidobacterium dentium</i> CH51
22	<i>Lactobacillus johnsonii</i> CH22	52	<i>Bifidobacterium infantis</i> CH52
23	<i>Lactobacillus brevis</i> CH23	53	<i>Bifidobacterium infantis</i> CH53
24	<i>Lactobacillus paracasei</i> CH24	54	<i>Bifidobacterium infantis</i> CH54
25	<i>Lactobacillus kimchi</i> CH25	55	<i>Bifidobacterium longum</i> CH55
26	<i>Lactobacillus gasseri</i> CH26	56	<i>Bifidobacterium longum</i> CH56
27	<i>Lactobacillus paracasei</i> CH27	57	<i>Bifidobacterium longum</i> CH57
28	<i>Lactobacillus pentosus</i> CH28	58	<i>Bifidobacterium longum</i> CH58
29	<i>Lactobacillus pentosus</i> CH29	59	<i>Bifidobacterium longum</i> CH59
30	<i>Lactobacillus reuteri</i> CH30	60	<i>Bifidobacterium longum</i> CH60

Among the strains shown in Table 1 above, *Lactobacillus brevis* CH23 was a gram-positive anaerobic bacillus, did not form spores, and could survive even under aerobic conditions. Furthermore, *Lactobacillus brevis* CH23 survived at 10 to 42°C and was an acid-resistant strain stable at pH 2 for 2 hours. Furthermore, *Lactobacillus brevis* CH23 survived even in 2% sodium chloride solution and actively produced glucosidase. In addition, to chemically classify *Lactobacillus brevis* CH23, the 16S rDNA thereof was analyzed, and as a result, it was shown that *Lactobacillus brevis* CH23 had a nucleotide sequence of SEQ ID NO: 1. The 16S rDNA nucleotide sequence of *Lactobacillus brevis* CH23 was identified by BLAST in the Genbank (<http://www.ncbi.nlm.nih.gov/>), and as a result, a *Lactobacillus brevis* strain having the same 16S rDNA nucleotide sequence as that of *Lactobacillus brevis* CH23 was not found, and *Lactobacillus brevis* CH23 showed a homology of 99% with the 16S rDNA sequence of *Lactobacillus brevis* strain FJ004.

Among the strains shown in Table 1 above, *Lactobacillus johnsonii* CJLJ103 was a gram-positive anaerobic bacillus, did not form spores, and could survive under aerobic conditions. Furthermore, *Lactobacillus johnsonii* CJLJ103 survived stably at a temperature of 10 to 45°C, and was an acid-resistant strain stable in pH 2 for 2 hours. Moreover, *Lactobacillus johnsonii* CJLJ103 actively produced glucosidase, but did not produce β -glucuronidase. In addition, to chemically classify *Lactobacillus johnsonii* CJLJ103, the 16S rDNA thereof was analyzed, and as a result, it was shown that *Lactobacillus*

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johnsonii CJLJ103 had a nucleotide sequence of SEQ ID NO: 2. The 16S rDNA nucleotide sequence of *Lactobacillus johnsonii* CJLJ103 was identified by BLAST in Genebank (<http://www.ncbi.nlm.nih.gov/>), and as a result, a
5 *Lactobacillus johnsonii* strain having the same 16S rDNA nucleotide sequence as that of *Lactobacillus johnsonii* CJLJ103 was not found, and *Lactobacillus johnsonii* CJLJ103 showed a homology of 99% with the 16S rDNA sequence of *Lactobacillus johnsonii* strain JCM 2012.

10 Among the strains shown in Table 1 above, *Bifidobacterium longum* CH57 was a gram-positive anaerobic bacillus, did not form spores, and showed very low viability under aerobic conditions. Furthermore, *Bifidobacterium longum* CH57 was thermally unstable. Furthermore, *Bifidobacterium longum* CH57
15 actively produced glucosidase, but did not produce β -glucuronidase. In addition, to chemically classify *Bifidobacterium longum* CH57, the 16S rDNA thereof was analyzed, and as a result, it was shown that *Bifidobacterium longum* CH57 had a nucleotide sequence of SEQ ID NO: 3. The 16S rDNA
20 nucleotide sequence of *Bifidobacterium longum* CH57 was identified by BLAST in the Genebank (<http://www.ncbi.nlm.nih.gov/>), and as a result, a *Bifidobacterium longum* strain having the same 16S rDNA nucleotide sequence as that of *Bifidobacterium longum* CH57 was
25 not found, and *Bifidobacterium longum* CH57 showed a homology of 99% with the 16S rDNA sequence of *Bifidobacterium longum* strain CBT-6.

In addition, among the physiological characteristics of *Lactobacillus brevis* CH23, *Lactobacillus johnsonii* CJLJ103 and *Bifidobacterium longum* CH57, the carbon source utilization was analyzed using a sugar fermentation by an API kit (model: API 50 CHL; manufactured by BioMerieux's, USA). Table 2 below shows the results of analyzing the carbon source utilization of *Lactobacillus brevis* CH23; Table 3 below shows the results of analyzing the carbon source utilization of *Lactobacillus johnsonii* CJLJ103; and Table 4 below shows the results of analyzing the carbon source utilization of *Bifidobacterium longum* CH57. In Tables 2, 3 and 4, "+" indicates the case in which carbon source utilization is positive; "-" indicates the case in which carbon source utilization is negative; and "±" indicates the case in which carbon source utilization is ambiguous. As shown in Tables 2, 3 and 4 below, *Lactobacillus brevis* CH23, *Lactobacillus johnsonii* CJLJ103 and *Bifidobacterium longum* CH57 showed carbon source utilization different from that of other strains of the same species with respect to some carbon sources.

Table 2

Carbon source	Strain name		Carbon source	Strain name	
	<i>L. brevis</i> ¹⁾	<i>L. brevis</i> CH23		<i>L. brevis</i> ¹⁾	<i>L. brevis</i> CH23
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- β -D-xylopyranoside	-	-	melezitose	+	-
D-galactose	+	-	raffinose	-	-
D-glucose	+	+	starch	-	-
D-fructose	+	+	glycogen	-	-
D-mannose	+	-	xylitol	-	-
L-sorbose	-	-	gentiobiose	+	-
L-rhamnose	-	-	D-turanose	+	-
dulcitol	+	-	D-lyxose	-	-
inositol	-	-	D-tagatose	+	-
mannitol	+	-	D-fucose	-	-
sorbitol	+	-	L-fucose	-	-
α -methyl-D-mannoside	-	-	D-arabitol	-	-
α -methly-D-glucoside	-	-	L-arabitol	-	-
N-acetyl-glucosamine	+	$\pm$	gluconate	+	$\pm$
amygdalin	+	-	2-keto-gluconate	-	-
arbutin	+	-	5-keto-gluconate	-	+
esculin	+	+			

1) Suriasih K., Aryanta WR, MahardikaG, Astawa NM. Microbiological and Chemical Properties of Kefir Made of Bali Cattle Milk. Food Science and Quality Management 2012;6:112-22.

Table 3

Carbon source	Strain name		Carbon source	Strain name	
	<i>L. johnsonii</i> ²⁾	<i>L. johnsonii</i> CJLJ103		<i>L. johnsonii</i> ²⁾	<i>L. johnsonii</i> CJLJ103
glycerol	-	-	salicin	-	-
erythritol	-	-	cellobiose	+	-
D-arabinose	-	-	maltose	-	+
L-arabinose	-	-	lactose	-	+
D-ribose	-	-	melibiose	+	-
D-xylose	-	-	sucrose	+	+
L-xylose	-	-	trehalose	+	-
D-adonitol	-	-	inulin	-	-

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methyl- β -D-xylopyranoside	-	-	melezitose	-	-
D-galactose	-	-	raffinose	+	-
D-glucose	-	+	starch	-	-
D-fructose	-	+	glycogen	-	-
D-mannose	+	+	xylitol	-	-
L-sorbose	-	-	gentiobiose	-	+
L-rhamnose	-	-	D-turanose	-	-
dulcitol	-	-	D-lyxose	-	-
inositol	-	-	D-tagatose	-	-
mannitol	-	-	D-fucose	-	-
sorbitol	-	-	L-fucose	-	-
α -methyl-D-mannoside	-	-	D-arabitol	-	-
α -methly-D-glucoside	-	-	L-arabitol	-	-
N-acetyl-glucosamine	+	+	gluconate	-	-
amygdalin	-	-	2-keto-gluconate	-	-
arbutin	-	-	5-keto-gluconate	-	-
esculin	-	-			

2) Pridmore RD, Berger B, Desiere F, Vilanova D, Barretto C, Pittet AC, Zwahlen MC, Rouvet M, Altermann E, Barrangou R, Mollet B, Mercenier A, Klaenhammer T, Arigoni F, Schell MA. The genome sequence of the probiotic intestinal bacterium *Lactobacillus johnsonii* NCC 533. Proc Natl Acad Sci U S A. 2004 Feb 24;101(8):2512-7.

Table 4

Carbon source	Strain name		Carbon source	Strain name	
	<i>B. longum</i> ³⁾	<i>B. longum</i> CH57		<i>B. longum</i> ³⁾	<i>B. longum</i> CH57
glycerol	±	-	salicin	±	-
erythritol	-	-	cellobiose	±	±
D-arabinose	-	-	maltose	-	-
L-arabinose	-	-	lactose	-	-
D-ribose	±	-	melibiose	-	-

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D-xylose	-	-	sucrose	+	±
L-xylose	-	-	trehalose	±	-
D-adonitol	-	-	inulin	-	-
methyl-β-D-xylopyranoside	-	-	melezitose	-	-
D-galactose	+	+	raffinose	-	-
D-glucose	+	+	starch	-	-
D-fructose	+	+	glycogen	-	-
D-mannose	-	-	xylitol	-	-
L-sorbose	-	-	gentiobiose	-	-
L-rhamnose	-	-	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	-	-
esculin	-	-			

3) Lukacova D, Karovucova J, Greifova M, Greif G, Sovcikova A, Kohhajdova Z. In vitro testing of selected probiotic characteristics of *Lactobacillus plantarum* and *Bifidobacterium longum*. Journal of Food and Nutrition Research 2006; 45: 77-83.

5 (4) Information on Deposition of Lactic Acid Bacteria

The present inventors deposited *Lactobacillus brevis* CH23 with the Korean Culture Center of Microorganisms (address: Yurim Building, 45, Hongjenae 2ga-gil, Seodaemun-gu, Seoul, Korea), an international depositary authority, on September 1,
10 2015 under accession number KCCM 11762P. Furthermore, the

present inventors deposited *Lactobacillus johnsonii* CJLJ103 with the Korean Culture Center of Microorganisms (address: Yurim Building, 45, Hongjenae 2ga-gil, Seodaemun-gu, Seoul, Korea), an international depositary authority, on September 1, 5 2015, under accession number KCCM 11763P. Furthermore, the present inventors deposited *Bifidobacterium longum* CH57 with the Korean Culture Center of Microorganisms (address: Yurim Building, 45, Hongjenae 2ga-gil, Seodaemun-gu, Seoul, Korea)), an international depositary authority, on September 1, 2015 10 under accession number KCCM 11764P.

2. Evaluation of the Effect of Lactic Acid Bacteria on Alleviation of Intestinal Damage or Intestinal Permeability

In order to evaluate the effect of the lactic acid bacteria isolated from kimchi or human feces, on the 15 alleviation of intestinal damage or internal permeability, the antioxidant activity, lipopolysaccharide (LPS) production inhibitory activity, β -glucuronidase (harmful intestinal enzyme) inhibitory activity, tight junction protein expression inducement activity and memory improvement activity of the 20 lactic acid bacteria were measured.

(1) Experimental Methods

*** Antioxidant activity**

DPPH (2,2-diphenyl-1-picrylhydrazyl) was dissolved in ethanol to a concentration of 0.2 mM to prepare a DPPH solution. 25 A lactic acid bacteria suspension (1×10^8 CFU/ml) or a vitamin C solution (1 g/ml) was added to 0.1 ml of the DPPH solution and cultured at 37°C for 20 minutes. The culture was centrifuged

at 3000 rpm for 5 minutes, and the supernatant was collected. Next the absorbance of the supernatant at 517 nm was measured, and the antioxidant activity of the lactic acid bacteria was calculated.

5 * Lipopolysaccharide (LPS) production inhibitory activity

Pathogenic bacteria, such as *Escherichia coli*, *Klebsiella pneumonia* and *Proteus mirabilis* were separated from the elderly subject and cultured. Next, the pathogenic bacteria (1×10^5 CFU respectively) and lactic acid bacteria (1×10^5 CFU) were

10 transplanted into 10 ml of sterile general anaerobic medium (GAM broth; Nissui Pharmaceutical, Japan) and anaerobically cultured for 24 hours.

Next, the culture was sonicated for about 1 hour to disrupt the outer cell membrane of the bacteria, and
15 centrifuged at $5000 \times g$, and the supernatant was collected. Next, the content of LPS (lipopolysaccharide) (which is a typical endotoxin) in the supernatant was measured by a LAL (Limulus Amoebocyte Lysate) assay kit (manufactured by Cape Cod Inc., USA). In addition, in order to evaluate the *E. coli*
20 proliferation inhibitory activity of the lactic acid bacteria, the culture obtained through the same experiment as described above was diluted 1000-fold and 100000-fold and cultured in DHL medium, and then the number of *E. coli*, *Klebsiella pneumonia* and *Proteus mirabilis* was counted.

25 * β -glucuronidase inhibitory activity

0.1 ml of 0.1 mM p-nitrophenyl- β -D-glucuronide solution, 0.2 ml of 50 mM phosphate buffered saline and 0.1 ml of a

lactic acid bacteria suspension (prepared by suspending of a lactic acid bacteria culture in 5 ml of physiological saline) were placed in a reactor and subjected to an β -glucuronidase enzymatic reaction, and 0.5 ml of 0.1 mM NaOH solution was added to stop the reaction. Next, the reaction solution was centrifuged at 3000 rpm for 5 minutes, and the supernatant was collected. Then, the absorbance of the supernatant at 405 nm was measured.

* Tight junction protein expression inducement activity

Caco2 cells obtained from the Korean Cell Line Bank were cultured in RPMI 1640 medium for 48 hours, and then the cultured Caco2 cells were dispensed to each well of a 12-well plate at a density of 2×10^6 cells/well. Next, each well was treated with 1 μ g of LPS (lipopolysaccharide) or a combination of 1 μ g of LPS (lipopolysaccharide) and 1×10^4 CFU of lactic acid bacteria and incubated for 24 hours. Next, the cultured cells were collected from each well, and the expression level of tight junction protein ZO-1 in the cells was measured by an immunoblotting method.

*Memory improvement activity

SH-SY5Y cells obtained from the Korean Cell Line Bank were cultured in DMEM medium, in which 10% of FBS and 1% of antibiotics were added, and dispensed to each well of a 12-well plate at a density of 2×10^6 cells/well. Next, along with lactic acid bacteria (1×10^4 CFU/ml), LPS (lipopolysaccharide), separated from *Proteus mirabilis*, was added to each well at the

concentration of 0.2 mg/ml and cultured, and then the level of inhibition on NF- κ B(nuclear factor kappa-light-chain-enhancer of activated B cells) activity and expression level of α -synuclein were measured by the immunoblotting method. NF- κ B is known as a substance that causes tissue damage by inflammation reaction and aging-related diseases like Alzheimer's disease, while α -synuclein is known as a substance that causes Parkinson's disease.

(2) Experimental Results

The antioxidant activity, lipopolysaccharide (LPS) production inhibitory activity, β -glucuronidase inhibitory activity and tight junction protein expression inducement activity of the lactic acid bacteria isolated from kimchi or human feces were measured, and the results of the measurement are shown in Tables 5, 6 and 7 below. As shown in Tables 5, 6 and 7 below, *Lactobacillus curvatus* CH5, *Lactobacillus sakei* CH11, *Lactobacillus brevis* CH23, *Lactobacillus johnsonii* CJLJ103, *Bifidobacterium pseudocatenulatum* CH38 and *Bifidobacterium longum* CH57 had excellent antioxidant activity, strongly inhibited lipopolysaccharide (LPS) production and β -glucuronidase activity, strongly induced the expression of tight junction protein, strongly inhibited the NF- κ B activity, and strongly inhibited the expression of α -synuclein. These lactic acid bacteria have an excellent antioxidant effect, have an excellent effect of inhibiting the enzymatic activity of intestinal flora's harmful bacteria associated with inflammation and carcinogenesis, inhibit the production of

endotoxin LPS (lipopolysaccharide) produced by intestinal flora's harmful bacteria, and induce the expression of tight junction protein. Thus, these lactic acid bacteria can improve intestinal permeability syndrome. Also, the lactic acid bacteria can alleviate Alzheimer's disease and Parkinson's disease, as the lactic acid bacteria inhibit not only the production of LPS(lipopolysaccharide), an endotoxin of intestinal microorganisms, such as *K. pneumoniae*, *E. coli* and *P. mirabilis*, associated with Alzheimer's disease or Parkinson's disease, but also the production or activity of substances inducing neurodegeneration. In particular, the lactic acid bacteria are expected to bring a synergistic effect through various functions, such as alleviation of Intestinal Permeability Syndrome and inhibition on the production of endotoxin and the activity or production of substances inducing neurodegenerative.

Table 5

Cont No.	Strain name	Antioxidant activity	β -glucuronidase inhibitory activity	tight junction protein expression inducement activity	SH-SY5Y cell		LPS production inhibitory		
					inhibition on NF- κ B	inhibition on α -synuclein	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>P. mirabilis</i>
1	<i>L. acidophilus</i> CH1	+	+	-	-	-	-	-	-
2	<i>L. acidophilus</i> CH2	+	+	-	-	-	+	+	+

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3	<i>L. acidophilus CH3</i>	+	+	-	-	-	+	+	+
4	<i>L. brevis CH4</i>	+	+	-	++	+	-	+	+
5	<i>L. curvatus CH5</i>	+++	+	++	++	+	+	+	+
6	<i>L. brevis CH6</i>	+	+	-	+	-	-	-	-
7	<i>L. casei CH7</i>	+	+	-	-	-	-	-	-
8	<i>L. plananturum CH8</i>	+	+	-	+	-	+	+	+
9	<i>L. sakei CH9</i>	-	+	-	-	-	-	-	-
10	<i>L. curvatus CH10</i>	-	+	-	+	-	-	-	-
11	<i>L. sakei CH11</i>	+++	+	++	++	++	++	+	+
12	<i>L. curvatus CH12</i>	-	+	+	+	+	-	-	-
13	<i>L. plantarum CH13</i>	-	+	-	+	-	-	-	-
14	<i>L. fermentum CH14</i>	-	+	-	-	+	-	-	-
15	<i>L. fermentum CH15</i>	+++	+	-	-	-	+	+	+
16	<i>L. gasserii CH16</i>	+	+	-	-	-	+	+	+
17	<i>L. paracasei CH17</i>	+	+	-	+	-	-	-	-
18	<i>L.</i>	+	+	-	-	-	-	-	-

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	<i>helveti</i> <i>cus</i> CH18								
19	<i>L.</i> <i>helveti</i> <i>cus</i> CH19	+	+	-	-	-	-	-	-
20	<i>L.</i> <i>johnson</i> <i>ii</i> CH20	+	+	+	+	+	-	-	-

Table 6

Cont No.	Strain name	Antiox idant activi ty	β - glucuro nidase inhibit ory activit y	tight junction protein expression inducement activity	SH-SY5Y cell		LPS production inhibitory		
					inhibi tion on NF- kB	inhibi tion on α - synucl ein	<i>K.</i> <i>pneup</i> <i>omiae</i>	<i>E.</i> <i>col</i> <i>i</i>	<i>P.</i> <i>mirab</i> <i>ilis</i>
21	<i>L.</i> <i>johnson</i> <i>ii</i> CH21	+	+	+	+	++	+	+	+
22	<i>L.</i> <i>johnson</i> <i>ii</i> CH22	+	+	+	+	+	-	-	-
23	<i>L.</i> <i>brevi</i> <i>s</i> CH23	+++	+	++	++	+	++	++	++
24	<i>L.</i> <i>paracas</i> <i>ei</i> CH24	+	+	-	-	-	-	-	-
25	<i>L.</i> <i>kimchi</i> CH25	+	+	-	-	-	-	-	-
26	<i>L.</i> <i>gasser</i> <i>i</i> CH26	+	+	-	-	-	-	-	-
27	<i>L.</i> <i>paracas</i> <i>ei</i> CH27	+	+	+	+	-	-	-	-
28	<i>L.</i> <i>pentosu</i> <i>s</i> CH28	+	+	-	+	+	-	-	-
29	<i>L.</i> <i>pentosu</i>	+	+	-	-	-	-	-	-

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	<i>s</i> CH29								
30	<i>L.</i> <i>reuteri</i> CH30	+	-	-	-	-	-	-	-
31	<i>L.</i> <i>sakei</i> CH31	-	+	+	+	+	-	-	-
32	<i>L.</i> <i>johnsonii</i> CJLJ103	+++	+	++	++	+++	++	++	+++
33	<i>L.</i> <i>sakei</i> CH33	+	+	+	-	+	-	-	-
34	<i>L.</i> <i>sakei</i> CH34	+	+	+	-	+	+	+	+
35	<i>L.</i> <i>plantarum</i> CH35	+	+	+	+	+	+	+	+
36	<i>L.</i> <i>sanfranciscensis</i> CH36	+	+	+	+	+	+	-	-
37	<i>B.</i> <i>pseudocatenulatum</i> CH37	-	+	+	+	+	-	-	-
38	<i>B.</i> <i>pseudocatenulatum</i> CH38	+++	+	++	+	++	+	+	+
39	<i>B.</i> <i>adolescentis</i> CH39	-	+	+	+	+	-	-	-
40	<i>B.</i> <i>adolescentis</i> CH40	-	+	+	+	+	+	+	+

Table 7

Cont No.	Strain name	Antioxidant	β -	tight junction	SH-SY5Y cell	LPS production inhibitory
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			glucuro nidase inhibit ory	protein expression inducement	inhibi tion on NF- κB	inhibi tion on α- synucl ein	<i>K.</i> <i>pneup</i> <i>oniae</i>	<i>E.</i> <i>col</i> <i>i</i>	<i>P.</i> <i>mirab</i> <i>ilis</i>
41	<i>B.</i> <i>adolesc</i> <i>entis</i> CH41	+	+	+	+	+	+	+	+
42	<i>B.</i> <i>animali</i> <i>s</i> CH42	+	+	-	+	-	-	-	-
43	<i>B.</i> <i>animali</i> <i>s</i> CH43	+	+	-	+	-	-	-	-
44	<i>B.</i> <i>bifidum</i> CH44	+	+	-	+	-	-	-	-
45	<i>B.</i> <i>bifidum</i> CH45	+	+	-	+	-	-	-	-
46	<i>B.</i> <i>breve</i> CH46	+	-	-	-	-	-	+	+
47	<i>B.</i> <i>breve</i> CH47	+	+	+	++	+	-	-	-
48	<i>B.</i> <i>breve</i> CH48	+	+	+	+	+	-	-	-
49	<i>B.</i> <i>catenul</i> <i>atum</i> CH49	+	+	++	+	++	-	-	-
50	<i>B.</i> <i>catenul</i> <i>atum</i> CH50	-	+	-	+	-	-	-	-
51	<i>B.</i> <i>dentium</i> CH51	+	-	-	-	-	-	-	-
52	<i>B.</i> <i>infanti</i> <i>s</i> CH52	-	+	-	-	-	-	-	-
53	<i>B.</i> <i>infanti</i> <i>s</i> CH53	-	+	-	+	-	-	-	-

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54	<i>B. infantis</i> CH54	+	+	-	-	-	-	-	-
55	<i>B. longum</i> CH55	+	+	+	+	+	-	-	-
56	<i>B. longum</i> CH56	+++	+	+	+	+	++	-	-
57	<i>B. longum</i> CH57	+++	+	++	+	++	+++	+	++
58	<i>B. longum</i> CH58	+	+	+	+	+	+	+	+
59	<i>B. longum</i> CH59	+	+	+	+	+	+	+	+
60	<i>B. longum</i> CH60	+	-	+	-	+	-	-	-

* The final concentration of lactic acid bacteria in measurement of antioxidant activity: 1×10^4 CFU/ml; the concentration of lactic acid bacteria added for measurement of beta-glucuronidase inhibitory activity and lipopolysaccharide (LPS) production inhibitory activity: 1×10^4 CFU/ml; the concentration of lactic acid bacteria in measurement of tight junction protein expression inducement activity: 1×10^4 CFU/ml.

* Criteria for measurement of various activities of lactic acid bacteria: very strongly (+++; >90%); strongly (++; >60-90%); weakly (+; >20-60%); not or less than 20% (-; <20%).

3. Measurement of improvement effect of lactic acid bacteria for cognitive ability by using model animals with induced memory damage

(1) Selection of lactic acid bacteria for experiment to measure improvement effect for cognitive ability

The following 13 types of lactic acid bacteria were selected as lactic acid bacteria for the experiment to measure improvement effect for cognitive ability among a total of 60 types of lactic acid bacteria isolated from Kimchi or human feces.

* *Lactobacillus acidophilus* CH3, *Lactobacillus curvatus* CH5, *Lactobacillus sakei* CH11, *Lactobacillus fermentum* CH15, *Lactobacillus johnsonii* CH21, *Lactobacillus brevis* CH23, *Lactobacillus johnsonii* CJLJ103, *Lactobacillus plantarum* CH35, *Bifidobacterium pseudocatenulatum* CH38, *Bifidobacterium adolescentis* CH41, *Bifidobacterium longum* CH56, *Bifidobacterium longum* CH57, *Bifidobacterium longum* CH59

(2) Passive Avoidance test and the result

In the Passive Avoidance test apparatus, which is divided into the first and second space, there is a guillotine-like door that connects the two spaces. By using lightings, the first space was kept bright while the second space was kept dark. On the floor of the second space that was kept dark was placed an electric grid that flowed 0.5 mA of electric shock for three seconds in the case of the animal moving to the dark space.

The selected 13 types of lactic acid bacteria were each suspended in physiological saline at the concentration of 1×10^{10} CFU/mL, and then the suspension was administered to mice of the test animal (5-week-old ICR male mice from Raonbio), at the dosage of 0.1 mL (equivalent to 1×10^9 CFU of lactic acid

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bacteria) on a daily basis for three days. Also, 0.1ml of physiological saline were administered to the mice corresponding to the normal group and memory impairment group on a daily basis for three days. The mice corresponding to the positive control group were administered with 5 mg/kg (body weight) of the positive control drug, donepezil (treatment for Alzheimer's disease and Alzheimer's disease-type dementia), on a daily basis for three days. The number of mice per the experimental group was 9.

On the second day after administering the drug, the above mice were placed in the first space which was bright, observed for 20 seconds, and allowed to move into the second space that was kept dark by opening the guillotine-like door. The mice, which did not move to the second space for 60 seconds after the door was opened, were excluded from the experiment. Once the mice move to the second space, the guillotine-like door is closed, and the mice receive the electric shock at 0.5mA through the grid on the floor for three seconds. The mice are forced to remember the shock.

On the third day after administering the drug, an hour after administering lactic acid bacteria or positive control drug, 1 mg/kg (body weight) of scopolamine (cholinergic blocking drug inducing cognitive ability disorder or memory damage), dissolved in distilled water, was administered in the abdomen while the normal group was administered with physiological saline in the abdomen. The experiment was conducted by using Passive Avoidance experiment apparatus after 30 minutes of administration of scopolamine. With 10 seconds of observation,

the guillotine-like door is opened, the time that the mice in each experimental group took to have all of their four feet inside the second space was measured up to 300 seconds, whose result is shown in Table 8. The result in Table 8 shows that longer latency time means better performance in learning passive avoidance and recovering short-term memory.

Table 8

Group	Latency time; sec
Normal group	240.2
Memory damage group only administered scopolamine	22.6
Positive group administered scopolamine and donepezil	66.4
Administered scopolamine and <i>Lactobacillus acidophilus</i> CH3	33.5
Administered scopolamine and <i>Lactobacillus curvatus</i> CH5	57.4
Administered scopolamine and <i>Lactobacillus sakei</i> CH11	45.0
Administered scopolamine and <i>Lactobacillus fermentum</i> CH15	56.7
Administered scopolamine and <i>Lactobacillus johnsonii</i> CH21	22.4
Administered scopolamine and <i>Lactobacillus brevis</i> CH23	61.2
Administered scopolamine and <i>Lactobacillus johnsonii</i> CJLJ103	71.0
Administered scopolamine and <i>Lactobacillus plantarum</i> CH35	44.6
Administered scopolamine and <i>Bifidobacterium pseudocatenulatum</i> CH38	49.6
Administered scopolamine and <i>Bifidobacterium adolescentis</i> CH41	35.6
Administered scopolamine and <i>Bifidobacterium longum</i> CH56	54.9

Administered scopolamine and <i>Bifidobacterium longum</i> CH57	62.1
Administered scopolamine and <i>Bifidobacterium longum</i> CH59	48.5

As shown in Table 8, when model animals, induced to have cognitive ability disorder or memory impairment by scopolamine, were administered with *Lactobacillus brevis* CH23, *Lactobacillus johnsonii* CJLJ103 or *Bifidobacterium longum* CH57, latency time significantly increased compared to the memory impairment group administered with only scopolamine, and especially, administration of *Lactobacillus johnsonii* CJLJ103 showed better effect than the administration of donepezil, a commercial treatment.

(3) Y-maze test and the result

Apparatus used in the Y-maze test consists of three arms with each being 42cm in length, 3cm in width and 12cm in height. The three arms, made from black polyvinyl resin, were placed at an angle of 120 degrees.

The selected 13 types of lactic acid bacteria were each suspended in physiological saline at the concentration of 1×10^{10} CFU/ml, and then the suspension was administered to mice of the test animal (5-week-old ICR male mice from Raonbio), at the dosage of 0.1ml (equivalent to 1×10^9 CFU of lactic acid bacteria) on a daily basis for three days. Also, 0.1ml of physiological saline were administered to the mice corresponding to the normal group and memory impairment group

on a daily basis for three days. The mice corresponding to the positive control group were administered with 5 mg/kg (body weight) of the positive control drug, donepezil (treatment for Alzheimer's disease and Alzheimer's disease-type dementia), on a daily basis for three days. The number of mice per experimental group was 9.

An hour after the final administration of lactic acid bacteria or positive control drug, 1 mg/kg (body weight) of scopolamine (cholinergic blocking drug inducing cognitive ability disorder or memory impairment), dissolved in distilled water, was administered in the abdomen while the normal group was administered with physiological saline in the abdomen. half an hour after the administration of scopolamine, the mouse was carefully placed in one of the three arms, A, B, and C, in the Y-maze and allowed to freely move for eight minutes, and then the arm in which the mouse moved was recorded. In the experiment, the arm was recorded as a case in which the test animal moved only when the entire body including the tail was inside the arm, or when the mouse moved back in. In the case of the mouse moving into each arm sequentially, one point (actual alteration) was allocated. Alteration behavior is defined as a case where the mouse moved in all of the three arms sequentially. The experimental result was calculated by the formula below (Sarter, M. et al., Psychopharmacology., 94, pp491-495, 1998), as shown in Table 9 below.

Spontaneous alteration (%)

= actual alternation / maximum alternation X 100

(maximum alteration: the total number of entries - 2)

Table 9 below shows that greater alternation behavior(unit:%) means better recovery of learning and spatial
5 memory.

Table 9

Group	Alternation behavior; %
Normal group	74.5
Memory damage group only administered scopolamine	49.9
Positive group administered scopolamine and donepezil	65.8
Administered scopolamine and <i>Lactobacillus acidophilus</i> CH3	53.7
Administered scopolamine and <i>Lactobacillus curvatus</i> CH5	51.3
Administered scopolamine and <i>Lactobacillus sakei</i> CH11	49.0
Administered scopolamine and <i>Lactobacillus fermentum</i> CH15	56.2
Administered scopolamine and <i>Lactobacillus johnsonii</i> CH21	53.5
Administered scopolamine and <i>Lactobacillus brevis</i> CH23	59.2
Administered scopolamine and <i>Lactobacillus johnsonii</i> CJLJ103	63.9
Administered scopolamine and <i>Lactobacillus plantarum</i> CH35	52.4
Administered scopolamine and <i>Bifidobacterium pseudocatenulatum</i> CH38	52.5
Administered scopolamine and <i>Bifidobacterium adolescentis</i> CH41	54.1

Administered scopolamine and <i>Bifidobacterium longum</i> CH56	55.2
Administered scopolamine and <i>Bifidobacterium longum</i> CH57	60.3
Administered scopolamine and <i>Bifidobacterium longum</i> CH59	55.5

As shown in Table 9, when model animals, induced to have cognitive ability disorder or memory impairment by scopolamine, were administered with *Lactobacillus brevis* CH23, *Lactobacillus johnsonii* CJLJ103 or *Bifidobacterium longum* CH57, alternation
5 behavior significantly increased compared to the memory impairment group administered with only scopolamine, and especially, administration of *Lactobacillus johnsonii* CJLJ103 showed excellent effect equivalent to the administration of donepezil, a commercial treatment.

10 **4. Preparation of red bean ferment by using lactic acid bacteria and measurement of improvement effect of the red bean ferment for cognitive ability**

(1) Preparation of red bean ferment

Lactobacillus johnsonii CJLJ103 was cultured in the
15 edible TS medium and centrifuged at 10,000gf for 20 minutes to yield *Lactobacillus johnsonii* CJLJ103 biomass. The yielded *Lactobacillus johnsonii* CJLJ103 biomass was washed with physiological saline twice and suspended in 100ml of physiological saline to prepare the suspension of *Lactobacillus*
20 *johnsonii* CJLJ103 biomass. Next, 10g of finely crushed red bean was added to 90ml of suspension of *Lactobacillus johnsonii* CJLJ103 biomass, which was then cultured for 24 hours to

ferment red bean. Next, the red bean ferment liquid was freeze-dried to yield red bean ferment.

(2) Passive Avoidance test and the result

Except the type and amount of administered drug, Passive
 5 Avoidance test was conducted in the same way as stated earlier
 herein, and the result is shown in Table 10 below. As shown in
 Table 10, administration of the red bean suspension or
Lactobacillus johnsonii CJLJ103 (daily dosage: 2×10^8 CFU/mouse)
 failed to significantly improve the damaged memory while the
 10 administration of the red bean ferment significantly improved
 the damaged memory.

Table 10

Group	Alternation behavior; %
Normal group	232.5
Memory damage group only administered scopolamine	23.8
Positive group administered scopolamine and donepezil	65.7
Administered scopolamine and red bean suspension	48.5
Administered scopolamine and <i>Lactobacillus johnsonii</i> CJLJ103	48.0
Administered scopolamine and red bean ferment	64.2

1) Red bean suspension was prepared by suspending red bean
 powder in physiological saline, and the daily dosage was 0.2g
 15 per mouse based on the amount of red bean powder.

2) *Lactobacillus johnsonii* CJLJ103 was administered after
 suspended in physiological saline, and the daily dosage was

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2×10⁸ CFU per mouse based on the amount of *Lactobacillus johnsonii* CJLJ103.

3) Red bean ferment was administered after suspended in physiological saline, and the daily dosage was 0.2g per mouse based on the amount of red bean ferment. The 0.2g of red bean ferment contained approximately 2×10⁸ CFU of *Lactobacillus johnsonii* CJLJ103.

(3) Y-maze test and the result

Except the type and amount of administered drug, Y-maze test was conducted in the same way as stated earlier herein, and the result is shown in Table 11 below. As shown in Table 11, administration of the red bean suspension or *Lactobacillus johnsonii* CJLJ103 (daily dosage : 2×10⁸ CFU/mouse) to the mouse failed to significantly improve the damaged memory while the administration of the red bean ferment significantly improved the damaged memory.

Table 11

Group	Alternation behavior; %
Normal group	75.2
Memory damage group only administered scopolamine	45.4
Positive group administered scopolamine and donepezil	63.2
Administered scopolamine and red bean suspension	52.4
Administered scopolamine and <i>Lactobacillus johnsonii</i> CJLJ103	57.6
Administered scopolamine and red bean ferment	63.3

1) Red bean suspension was prepared by suspending red bean powder in physiological saline, and the daily dosage was 0.2g per mouse based on the amount of red bean powder.

2) *Lactobacillus johnsonii* CJLJ103 was administered after
5 suspended in physiological saline, and the daily dosage was 2×10^8 CFU per mouse based on the amount of *Lactobacillus johnsonii* CJLJ103.

3) Red bean ferment was administered after suspended in physiological saline, and the daily dosage was 0.2g per mouse
10 based on the amount of red bean ferment. The 0.2g of red bean ferment contained approximately 2×10^8 CFU of *Lactobacillus johnsonii* CJLJ103.

**5. Measurement of improvement effect of lactic acid bacteria for cognitive ability by using model animals induced
15 to have memory impairment by *Escherichia coli***

(1) Inducement of memory impairment and administration of drug

Escherichia coli K20 separated from the animal with impaired memory was administered to the mouse to induce memory
20 impairment, and the improvement effect of lactic acid bacteria for cognitive ability was measured by conducting Y-maze test and Passive Avoidance test.

In more detail, *Escherichia coli* K20 was administered to a mouse (5-week-old ICR male mice from Raonbio) that was fed
25 for a week in the animal laboratory at the dosage of 1×10^9 CFU on a daily basis for five days to induce memory impairment. In the meantime, the normal group was administered with

physiological saline instead of *Escherichia coli* K20. From the next day of the final administration of *Escherichia coli* K20, lactic acid bacteria suspension (lactic acid bacteria suspended in physiological saline at the concentration of 1×10^{10} CFU/mL) was administered to the mouse induced to have memory impairment at the dosage of 0.1mL (equivalent to 1×10^9 CFU of lactic acid bacteria) on a daily basis for five days. Also, the normal group and memory impairment-induced group were administered with 0.1mL of physiological saline on a daily basis for five days. In addition, lactic acid bacteria suspension (lactic acid bacteria suspended in physiological saline at the concentration of 1×10^{10} CFU/mL) was administered to the normal group at the dosage of 0.1mL (equivalent to 1×10^9 CFU of lactic acid bacteria) on a daily basis for five days, which was used as a positive control group. The number of mice per experimental group was 9.

(2) Y-maze test

An hour after the final administration of drug, the mouse was carefully placed in one of the three arms, A, B, and C, in the Y-maze and allowed to freely move for eight minutes, and then the arm in which the mouse moved was recorded. In the experiment, the arm was recorded as a case in which the test animal moved only when the entire body including the tail was inside the arm, or when the mouse moved back in. In the case of the mouse moving into each arm sequentially, one point(actual

alteration) was allocated. Alteration behavior is defined as a case where the mouse moved in all of the three arms sequentially. The experimental result was calculated by the formula below (Sarter, M. et al., Psychopharmacology., 94, pp491-495, 1998).

Spontaneous alteration (%)

= actual alternation / maximum alternation X 100

(maximum alteration: the total number of entries - 2)

(3) Passive avoidance test

On the second day after administering the drug, the above mice were placed in the first space which was bright, observed for 20 seconds, and allowed to move into the second space that was kept dark by opening the guillotine-like door. The mice, which did not move to the second space for 60 seconds after the door was opened, were excluded from the experiment. Once the mice move to the second space, the guillotine-like door is closed, and the mice receive the electric shock at 0.5mA through the grid on the floor for three seconds. The mice are forced to remember the shock.

An hour after the final administration of drug, the experiment was conducted by using the Passive Avoidance test apparatus. With 10 seconds of observation, the guillotine-like door is opened, the time that the mice in each experimental group took to have all of their four feet inside the second space was measured up to 300 seconds.

(3) Measurement of the expression level of nerve growth-promoting factor

Two hours after the final administration of drug, hippocampus was separated from the mice of each experimental group, and the expression level of BDNF (Brain-Derived Neurotrophic Factor), known as a nerve growth-promoting factor, and the activity level of CREB (Cydic AMP Response Element-Binding), known as a memory-improving transcription factor, were measured.

(4) Experimental Result

FIG. 1 is a graph showing the impact that administration of *Lactobacillus johnsonii* CJLJ103 has on the Y-maze test of model animals induced to have memory impairment by *Escherichia coli* K20. FIG. 2 is a graph showing the impact that administration of *Lactobacillus johnsonii* CJLJ103 has on the Passive Avoidance test of model animals induced to have memory impairment by *Escherichia coli* K20 flora. FIG. 3 is a photo showing the impact that administration of *Lactobacillus johnsonii* CJLJ103 has on the expression level of nerve growth-promoting factor of model animals induced to have memory impairment by *Escherichia coli* K20.

From FIG. 1 to FIG. 3, "NOR" means the normal group, "NOR+CJLJ103" means the experimental group administered *Lactobacillus johnsonii* CJLJ103 in the normal group, "EC" is the memory impairment-induced group where only *Escherichia coli* K20 was administered, and "EC+CJLJ103" means the experimental group where memory impairment was induced by administering *Escherichia coli* K20 and then *Lactobacillus johnsonii* CJLJ103 was administered. As shown from FIG. 1 to FIG. 3,

administration of *Lactobacillus johnsonii* CJLJ103 to the normal group did not show significance while showing a slight existence of memory. On the other hand, administration of *Lactobacillus johnsonii* CJLJ103 to the mice induced to have memory impairment by *Escherichia coli* K20 significantly improved memory while improving the expression level of BDNF in hippocampus as well as the amount of activity (phosphorylation) of CREB.

6. Measurement of improvement effect of lactic acid bacteria for cognitive ability by using model animals with induced memory impairment by *Proteus mirabilis*

Proteus mirabilis K21 separated from the animal with damaged memory was administered to the mice to induce memory impairment (similar symptoms to Parkinson's disease), and Y-maze test was conducted to measure the improvement effect of lactic acid bacteria for cognitive ability.

In more detail, *Proteus mirabilis* K21 was administered to a mouse (5-week-old ICR male mice from Raonbio) that was fed for a week in the animal laboratory at the dosage of 1×10^9 CFU on a daily basis for five days to induce memory impairment. In the meantime, the normal group was administered with physiological saline instead of *Proteus mirabilis* K21. From the next day of the final administration of *Proteus mirabilis* K21, lactic acid bacteria suspension (lactic acid bacteria suspended in physiological saline at the concentration of 1×10^{10} CFU/mL) was administered to the mouse induced to have memory impairment at

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the dosage of 0.1ml (equivalent to 1×10^9 CFU of lactic acid bacteria) on a daily basis for five days. Also, the normal group and memory impairment-induced group were administered with 0.1ml of physiological saline on a daily basis for five days. In addition, donepezil (treatment for Alzheimer's disease and Alzheimer's disease-type dementia), a positive control drug, was administered to the positive control group at the dosage of 5 mg/kg (body weight) on a daily basis for five days. The number of mice per experimental group was 9.

An hour after the final administration of drug, the mouse was carefully placed in one of the three arms, A, B, and C, in the Y-maze and allowed to freely move for eight minutes, and then the arm in which the mouse moved was recorded. In the experiment, the arm was recorded as an arm in which the test animal moved only when the entire body including the tail was inside the arm, or when the mouse moved back in. In the case of the mouse moving into each arm sequentially, one point (actual alteration) was allocated. Alteration behavior is defined as a case where the mouse moved in all of the three arms sequentially. The experimental result was calculated by the formula below (Sarter, M. et al., Psychopharmacology., 94, pp491-495, 1998), as shown in Table 12 below.

Spontaneous alteration (%)

= actual alternation / maximum alternation X 100

(maximum alteration: the total number of entries - 2)

Table 12 below shows that greater alternation behavior(unit:%) means better recovery of learning and spatial memory.

Table 12

Group	Alternation behavior; %
Normal group	73.8
Memory damage group only administered <i>Proteus mirabilis</i> K21	46.4
Positive group administered <i>Proteus mirabilis</i> K21 and donepezil	58.6
Administered <i>Proteus mirabilis</i> K21 and <i>Lactobacillus acidophilus</i> CH3	52.5
Administered <i>Proteus mirabilis</i> K21 and <i>Lactobacillus curvatus</i> CH5	50.2
Administered <i>Proteus mirabilis</i> K21 and <i>Lactobacillus sakei</i> CH11	50.7
Administered <i>Proteus mirabilis</i> K21 and <i>Lactobacillus fermentum</i> CH15	55.5
Administered <i>Proteus mirabilis</i> K21 and <i>Lactobacillus johnsonii</i> CH21	54.7
Administered <i>Proteus mirabilis</i> K21 and <i>Lactobacillus brevis</i> CH23	53.3
Administered <i>Proteus mirabilis</i> K21 and <i>Lactobacillus johnsonii</i> CJLJ103	62.8
Administered <i>Proteus mirabilis</i> K21 and <i>Lactobacillus plantarum</i> CH35	54.1
Administered <i>Proteus mirabilis</i> K21 and <i>Bifidobacterium pseudocatenulatum</i> CH38	51.2
Administered <i>Proteus mirabilis</i> K21 and <i>Bifidobacterium adolescentis</i> CH41	55.3
Administered <i>Proteus mirabilis</i> K21 and <i>Bifidobacterium longum</i> CH56	54.8
Administered <i>Proteus mirabilis</i> K21 and	57.3

<i>Bifidobacterium longum</i> CH57	
Administered <i>Proteus mirabilis</i> K21 and <i>Bifidobacterium longum</i> CH59	54.9

As shown in Table 12, administration of *Lactobacillus johnsonii* CJLJ103 to model animals induced to have memory impairment by *Proteus mirabilis* K21 flora showed better effect than the administration of donepezil, a commercial treatment.

5 Although the present invention has been described above with reference to the examples, the scope of the present invention is not limited to these examples, and various modifications are possible without departing from the scope and idea of the present invention. Therefore, the scope of
10 protection of the present invention should be interpreted to include all embodiments falling within the appended claims.

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【CLAIMS】**【Claim 1】**

An isolated *Lactobacillus johnsonii* CJLJ103 (accession number: KCCM 11763P).

5 **【Claim 2】**

The isolated *Lactobacillus johnsonii* CJLJ103 (accession number: KCCM 11763P) according to claim 1, wherein the isolated *Lactobacillus johnsonii* utilizes D-glucose, D-fructose, D-mannose, N-acetyl-glucosamine, maltose, lactose, sucrose or
10 gentibiose as a carbon source.

【Claim 3】

A pharmaceutical composition for improving cognitive function, memory or learning responses in a subject with a degenerative brain disease or cognitive function disorder,
15 comprising the isolated *Lactobacillus johnsonii* CJLJ103 (accession number: KCCM 11763P) according to claim 1 or 2, a culture thereof, a lysate thereof or an extract thereof as an active ingredient.

【Claim 4】

20 A pharmaceutical composition for improving cognitive function, memory or learning responses in a subject with a degenerative brain disease or cognitive function disorder comprising a red bean ferment or an extract thereof as an

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active ingredient,

wherein the red bean ferment is a product fermented by the isolated *Lactobacillus johnsonii* CJLJ103 (accession number: KCCM 11763P) according to claim 1 or 2.

5 **【Claim 5】**

A food composition for improving cognitive function, memory or learning responses in a subject with a degenerative brain disease or cognitive function disorder comprising the isolated *Lactobacillus johnsonii* CJLJ103 (accession number: 10 KCCM 11763P) according to claim 1 or 2, a culture thereof, a lysate thereof or an extract thereof as an active ingredient.

【Claim 6】

A food composition for improving cognitive function, memory or learning responses in a subject with a degenerative 15 brain disease or cognitive function disorder comprising a red bean ferment or an extract of thereof as an active ingredient,

wherein the red bean ferment is a product fermented by the isolated *Lactobacillus johnsonii* CJLJ103 (accession number: KCCM 11763P) according to claim 1 or 2.

20 **【Claim 7】**

A method of improving cognitive function, memory or learning responses in a subject with a degenerative brain disease or cognitive function disorder comprising administering to a subject the isolated *Lactobacillus johnsonii* CJLJ103

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(accession number: KCCM 11763P) according to claim 1 or 2, pharmaceutical composition according to claim 3 or 4 or the food composition according to claim 5 or 6, thereby improving cognitive function, memory or learning responses in the subject.

5 **【Claim 8】**

 Use of the isolated *Lactobacillus johnsonii* CJLJ103 (accession number: KCCM 11763P) according to claim 1 or 2 or the food composition according to claim 5 or 6, in the manufacture of a medicament for improving cognitive function,
10 memory or learning responses in a subject with a degenerative brain disease or cognitive function disorder.

【Claim 9】

 The pharmaceutical composition according to claim 3 or 4, the food composition according to claim 5 or 6, the method
15 according to claim 7 or the use according to claim 8, wherein the degenerative brain disease is Alzheimer's disease, Parkinson's disease, Huntington's disease or dementia.

【Claim 10】

 The pharmaceutical composition according to claim 3 or 4,
20 the food composition according to claim 5 or 6, the method according to claim 7 or the use according to claim 8, wherein the dementia is selected from the group consisting of senile dementia, vascular dementia, Lewy body dementia, frontotemporal dementia, Alzheimer's disease-type dementia, Parkinson's

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disease-type dementia, Huntington's disease-type dementia, Creutzfeldt-Jacob disease-type dementia and Pick's disease-type dementia, normal pressure hydrocephalus-causing dementia and head injury-causing dementia.

5 **【Claim 11】**

 A food composition for improving memory or learning ability comprising the isolated *Lactobacillus johnsonii* CJLJ103 (accession number: KCCM 11763P) according to claim 1 or 2, a culture thereof, a lysate thereof or an extract thereof as an
10 active ingredient.

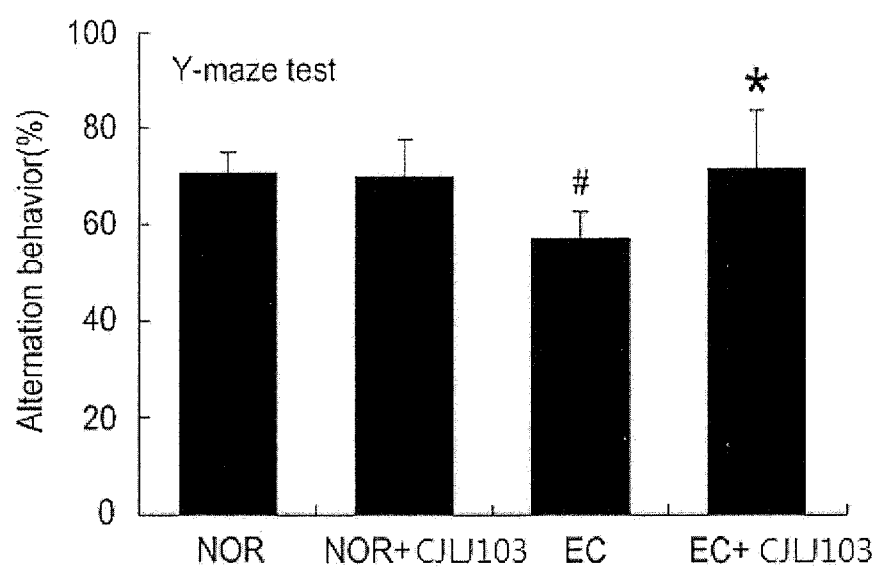
【Claim 12】

 The food composition for improving memory or learning ability comprising a red bean ferment or an extract thereof as an active ingredient,

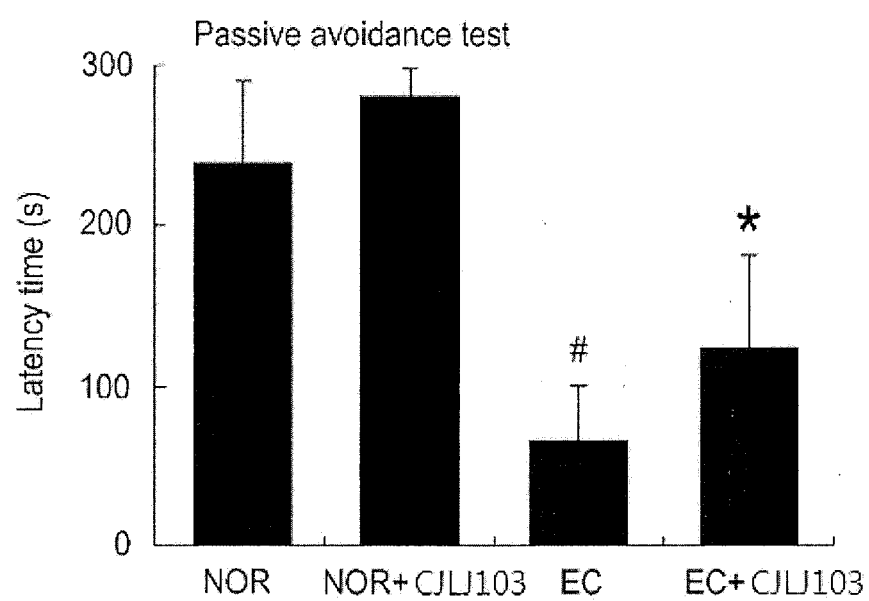
15 wherein the red bean ferment is a product fermented by the isolated *Lactobacillus johnsonii* CJLJ103 (accession number: KCCM 11763P) according to claim 1 or 2.

【DRAWINGS】

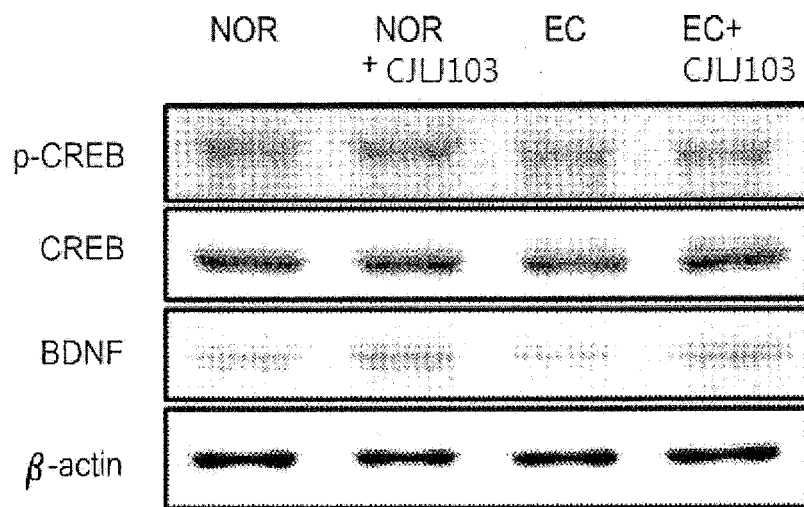
【Figure 1】



【Figure 2】



【Figure 3】



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<110> University-Industry Cooperation Group of Kyung Hee University

<120> Novel lactic acid bacteria and composition for preventing, improving or treating neurodegenerative diseases or cognitive dysfunction

<130> PCT- 16- 132

<150> KR 10- 2015- 0130124

<151> 2015- 09- 15

<150> KR 10- 2016- 0001312

<151> 2016- 01- 06

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