

Description

Title of the Invention: ANTIBACTERIAL PEPTIDE-INDUCING AGENT

Technical Field

[0001]

The present invention relates to an antibacterial peptide-inducing agent.

Background Art

[0002]

Mammals including human beings have become infected with pathogenic bacteria, fungi or viruses, and when these germs and viruses invade living bodies and establish themselves therein, they cause diarrhea, gastrointestinal diseases, high fever or the like. When these symptoms are serious, there may be cases where the infected bodies are led to death. Even by advanced medical treatment in this day and age, such infectious diseases of mammals are not eradicated yet, and hence they are major problems e.g. on the occasion when human beings want to lead healthy life.

[0003]

Elderly people, infants, children and the like who are weak in resistance against diseases, such as infection defense capability, tend to have higher incidences of those infectious diseases as compared to able-bodied adults. For example, because the infection self-defense mechanism deteriorates with age, elderly people are more likely to be infected with such normal bacterial flora (e.g. Staphylococcus aureus resident in nasal mucous membrane) as able-bodied adults don't contract them. For example, it has been said that infants and children are immature in immune system and weak in infection self-defense capability, and hence they are higher in probability of infectious disease incidence than able-bodied adults. In homes where those object people, which are weak in resistance against infectious diseases, spend time in groups, once an infectious disease occurs in some

individuals, protection against diffusion of the infectious disease compels the homes to take such a measure as to physically isolate the sufferers of the infectious disease or close the homes which sufferers of the infectious disease have stayed in or made a visit to.

[0004]

As a method for coping with an infectious disease, it is generally carried out to determine the cause of the infectious disease and then give treatment with a drug having antimicrobial activity (an antimicrobial medicine). However, when an antimicrobial medicine is administered to sufferers, it acts on not only the specific bacteria or viruses causing the infectious disease but also the so-called non-pathogenic intestinal flora including Bifidobacteria and normal bacterial flora; as a result, there is also a possibility of producing ill effects, such as diarrhea and infections caused by other pathogens. In addition, repeated or long-term administration of the same kind of antimicrobial medicine provides pathogenic bacteria, fungi or viruses with a chance that they themselves will build up tolerance to the antimicrobial medicine, and there may therefore be cases where treatment for the infectious disease becomes difficult.

[0005]

It is known that living things inherently secrete various kinds of peptides having antibacterial activities (antibacterial peptides) into bodies themselves in order to prevent pathogenic bacteria, fungi or viruses causing infectious diseases from invading and becoming established. Antibacterial peptides are peptides each of which is formed by combining 10 to 100 amino acids, and they have been isolated from a wide variety of living things including human beings. In addition, the antibacterial peptides are originated in hosts, and therefore they neither produce harmful aftereffects nor bring resistant bacteria into existence. In other words, if it is possible to promote secretion of antibacterial peptides in bodies, ideal protection against infection will be expected.

[0006]

Examples of antibacterial peptides originated in human beings include the Defensin family (e.g. α -Defensin, β -Defensin), Cathelicidin (LL-37), Dermcidin, LEAP-1 (Hepcidin), LEAP-2, lactoferrin and peptides belonging to the Regenerating Islet-derived family (Reg peptides). These antibacterial peptides are different in molecular structures and organs which secrete them, and also different in targeted substances on which they exert their antibacterial activities.

[0007]

If it becomes possible to make human beings artificially express or secrete those antibacterial peptides, prevention of infectious diseases and regression of symptoms will be expected. For example, there have been proposed the art of promoting secretion of human β -Defensin by using an organic acid as an active ingredient (Patent Document 1), the art of inducing gene expression of an antibacterial peptide by using as an active ingredient an insoluble fraction derived from yeast (Patent Document 2), the art of inducing expression of an antibacterial peptide by using as an active ingredient a yeast-derived mannan-containing ingredient (Patent Document 3), the art of inducing expression of antibacterial peptides by using as an active ingredient the concentrated yogurt for HI-CHEW, extract of a sweet alcoholic drink made from sake lees, unrefined Shochu, salted rice-bran paste concentrate or the like (Patent Document 4), the art of inducing secretion of an antibacterial peptide, such as α -Defensin, by using as active ingredients sterol glycosides derived from sprouted brown rice and/or brown rice bran (Patent Document 5) and the art of inducing secretion of an antibacterial peptide, such as α -Defensin, by using resveratrol as an active ingredient (Patent Document 6).

Documents Relating to Background Art

Patent Documents

[0006]

Patent Document 1: WO 2005/027893

Patent Document 2: JP-A-2003-000262

Patent Document 3: JP-A-2006-241023

Patent Document 4: JP-A-2005-270117

Patent Document 5: JP-A-2013-074821

Patent Document 6: J-P-A-2013-237643

Summary of the Invention

Problem that the Invention is to Solve

[0009]

A problem that the invention is to solve consists in providing an agent for inducing internal secretion of antibacterial peptides which can exert the biological self-defense mechanism present in a human body with safety and no harmful aftereffects.

Means for Solving the Problems

[0010]

In view of the problem mentioned above, we, the present inventors, have pursued intensive studies, and have obtained the following findings. To be more specific, we have found that the foregoing problem can be solved by demonstrating that intake of lactic acid bacteria allowed induction of gene expression of Defensins and Reg peptides. In addition, the lactic acid bacteria as the active ingredient in the invention and materials as their generic concept, namely lactic acid bacteria fermented materials, have been habitually eaten as fermentation food and the like for many years, and therefore the present agent for inducing internal secretion of antibacterial peptides is safe and extremely low in risk on harmful aftereffects.

[0011]

Further, intake of the present agent for inducing internal secretion of antibacterial peptides causes *in vivo* induction of the antibacterial peptides, and thereby it can be expected to maintain and enhance immunity due to prophylaxis of various infectious diseases, protect against aging and extend healthy life. Among these effects, it is to be expected in particular to prevent and/or treat infection diseases caused by opportunistic pathogens, infectious diseases caused by pathogenic bacteria and fungi, *Helicobacter pylori* infection, bacteria translocation, persistent inflammation of intestinal mucosa, change in balance of intestinal flora (e.g. collapse in balance of intestinal flora), diseases induced by these inflammations, Crohn's disease, inflammatory colitis such as ulcerative colitis, and excess inflammation and allergic symptoms resulting from induction of regulatory T cell differentiation. In addition, infants and children immature in immune function, elderly people declined in immunity and patients having affections can have expectations that the risk of infections will be lessened, inflammation will be soothed and allergic and chronic affections will be prevented or alleviated.

[0012]

As mentioned above, lactic acid bacteria have an action of inducing the gene expression of antibacterial peptides, specifically either Defensins or Reg peptides, or both. Accordingly, the invention provides an antibacterial peptide-inducing agent characterized by containing as an active ingredient either lactic acid bacteria or a material fermented with lactic acid bacteria, or both, and inducing gene expression of antibacterial peptides, or the invention provides a composition for induction of such an inducing agent, or the invention provides both the inducing agent and the composition. Further, the invention offers the use of the lactic acid bacteria in manufacturing the antibacterial peptide-inducing agent. In addition, the invention offers the use of the lactic acid bacteria in manufacturing a composition for inducing the antibacterial peptides.

[0013]

More specifically, the invention includes the following.

(1) An antibacterial peptide-inducing agent, characterized by containing lactic acid bacteria or a material fermented with lactic acid bacteria or both as active ingredients and inducing gene expression of antibacterial peptides.

(2) The antibacterial peptide-inducing agent as described in (1), wherein the antibacterial peptides are either Defensins or Regenerating Islet-derived family of peptides (Reg peptides), or both.

(3) The antibacterial peptide-inducing agent as described in (1) or (2), wherein the lactic acid bacteria are either lactic acid bacteria of the genus *Lactobacillus* or lactic acid bacteria of the genus *Streptococcus*, or both.

(4) An antibacterial peptide-inducing composition containing the antibacterial peptide-inducing agent as described in any of (1) to (3).

(5) The antibacterial peptide-inducing composition as described in (4), wherein the lactic acid bacteria are a combination of one or more than one strain selected from lactic acid bacteria of the genus *Lactobacillus* and one or more than one strain selected from lactic acid bacteria of the genus *Streptococcus*.

(6) The antibacterial peptide-inducing composition as described in (4), which contains at least one hundred million of lactic acid bacteria on a per-pack basis.

(7) The antibacterial peptide-inducing composition as described in any one of (1) to (6), wherein the lactic acid bacteria are viable bacteria.

(8) The antibacterial peptide-inducing composition as described in any one of (4) to (7), which is a food or medicine composition inducing gene expression of antibacterial peptides and having an infection prophylactic action.

(9) The food or medicine composition having an infection prophylactic action as

described in (8), which is taken in orally or enterally to induce antibacterial peptides.

(10) Use of lactic acid bacteria or a material obtained through fermentation with the lactic acid bacteria or a mixture thereof as active ingredients for gene expression of antibacterial peptides in manufacturing an antibacterial peptide-inducing agent.

(11) Use of lactic acid bacteria or a material obtained through fermentation with the lactic acid bacteria or a mixture thereof as active ingredients for gene expression of antibacterial peptides in manufacturing an antibacterial peptide-inducing composition containing an antibacterial peptide-inducing agent.

(12) Use of lactic acid bacteria or a material obtained through fermentation with the lactic acid bacteria or a mixture thereof as active ingredients for gene expression of antibacterial peptides in manufacturing a food or medicine composition containing an antibacterial peptide-inducing agent and having an infection prophylactic action.

Advantages of the Invention

[0014]

The invention is able to provide an antibacterial peptide internal secretion-inducing agent which can exert a biological self-defense mechanism present in the human body and is safe and free from harmful aftereffects.

Mode for Carrying Out the Invention

[0015]

The invention is described below in detail, but the invention should not be construed as being limited to the following individual modes.

[0016]

The term lactic acid bacteria as used in the invention is a generic name for all of taxonomically authorized ones as lactic acid bacteria or all of Bifidobacteria and related ones thereof, and have no restrictions as to bacterial species, bacterial strain and so on. In

addition, though there may be cases where lactic acid bacteria are classified as plant origin or animal origin according to their origins, both lactic acid bacteria of plant origin and those of animal origin may be used as the lactic acid bacteria in the invention. Of lactic acid bacteria, combinations of one or more than one strain selected from lactic acid bacteria of the genus *Lactobacillus* and one or more than one strain selected from lactic acid bacteria of the genus *Streptococcus* are favorable because their intake in the form of fermented milk, such as yogurt, has a track record in eating habits. Further, it is preferred that the lactic acid bacteria of the genus *Lactobacillus* be *Bulgaria* fungus or *Lactobacillus delbrueckii* ssp. *Bulgaricus* referred to as *Lactobacillus Bulgaricus* and the lactic acid bacteria of the genus *Streptococcus* be *Thermophilus* bacteria or *Streptococcus salivarius* ssp. *thermophilus* referred to as *Thermophilus*.

[0017]

The lactic acid bacteria relating to the invention can be used in a state of either viable or killed bacteria. In the invention, the lactic acid bacteria can also be used in a frozen or freeze dried form. Further, the lactic acid bacteria used in the invention may be only bacterial cells of the lactic acid bacteria, or in a form that ingredients other than the bacterial cells (e.g. a freeze protective agent, a freeze drying protective agent) are incorporated into bacterial cells of the lactic acid bacteria, or in a state that the lactic acid bacteria are dispersed into any of various media, such as starch and water, which have track records of being used as materials of medicines, food, drinks and so on.

[0018]

The present antibacterial peptide-inducing agent refers to an agent which can induce genes and the like in vivo when mammals including human beings take in the agent, resulting in production of antibacterial peptides derived from the genes. By the way, the wording “take in” used herein means that there is no restriction on the administration pathway so long

as the agent can be entered into the human body, and is intended to include all of known methods for administration, such as oral administration, tubal administration and enteral administration. Typical manners of intake are oral intake and enteral intake which are via the alimentary canal, and oral intake, especially in the form of food and drink, is preferable to enteral intake.

[0019]

The term antibacterial peptides as used in the invention refers to peptides having a function of defending living bodies against infection by pasteurization, sterilization or bacteriostasis of external bacteria which are not bacteria of internal origin. Examples of an antibacterial peptide include the Defensin family (e.g. α -Defensin, β -Defensin), Cathelicidin (LL-37), Dermcidin, LEAP-1 (Hepcidin), LEAP-2, lactoferrin and Reg peptides. The antibacterial peptides may be the relevant peptides themselves or in the form of their precursors to undergo activation by hydrolysis of their specified moieties.

[0020]

The term induction as used in the invention means that gene expression for expression of antibacterial peptides increases in vivo and, with the increase in the gene expression, antibacterial peptides are expressed. There is no restriction on the relevant genes, but preferred examples of such genes include genes of expressing Reg I, Reg II, Reg III α , Reg III β , Reg III γ and Defensins.

[0021]

Another embodiment of the invention is a Defensin family- and/or Reg peptide-related preventive and/or curative medicine for various diseases. By the wording of “various diseases” are collectively designated all of Defensin family- and/or Reg peptide-related diseases, symptoms, syndrome and those suggesting them, regardless of before or after the date based on the application date and /or priority date of the present invention, with examples

including diseases caused by opportunistic pathogens, infectious diseases caused by pathogenic bacteria, fungi and viruses, *Helicobacter pylori* infection, bacteria translocation, persistent inflammation of intestinal mucosa, change in balance of intestinal flora (e.g. collapse in balance of intestinal flora), diseases induced by these inflammations, Crohn's disease, inflammatory colitis such as ulcerative colitis, excess inflammation and allergic symptoms resulting from induction of regulatory T cell differentiation, and so on.

[0022]

Materials obtained by fermentation with the lactic acid bacteria according to the invention have no particular restrictions so long as they are materials fermented with lactic acid bacteria and habitually taken as medicines, food or the like by mammals including human beings. Of such materials, materials obtained by fermenting milk, soybeans, azuki beans, cereals, vegetables, meat, fish and mixtures thereof by the use of lactic acid bacteria can be exemplified.

[0023]

As an example of a material into which milk is fermented with lactic acid bacteria, mention may be made of the material prepared by compounding raw milk, a dairy product and raw water so as to contain 3.0 weight% of milk fat and 9.5 weight% of fat-free milk solid, optionally subjecting the compounded matter to homogenization treatment for controlling milk fat globules, performing heat sterilization under a sterilization condition comparable to retention of 65°C for 30 minutes, cooling the sterilized matter to a temperature in a range of 40°C to 45°C, and thereto adding as lactic acid bacteria of the genus *Lactobacillus* a mixed starter consisting of *Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus salinarum* subspecies *thermophilus*, and then fermenting the resulting admixture at a temperature in a range of 40°C to 45°C for 2 to 12 hours until the fermented matter has an acidity of from 0.6 to 1.2 weight% of lactic acid. At this time, there are no restrictions on

the strains of *Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus salinarum* subspecies *thermophilus*, and addition of lactic acid bacteria other than these two bacterial strains (e.g. genus *Lactobacillus*, genus *Bifidobacterium*, genus *Lactococcus*, genus *Leuconostoc*) and addition of other microorganisms, materials and additives useful for fermentation and health may be made arbitrarily. The term “raw milk” used herein is not limited to fresh milk obtained by milking cows, nanny goats, ewes, water buffalos or the like, or the so-called raw milk, and reconstituted milk having the same composition as the fresh or raw milk as mentioned above, but can also include enriched milk in which milk fat and/or nonfat milk solids (lactoprotein, lactose and minerals) are admixed and processed milk, such as low-fat milk and fat-free milk prepared by removing milk fat from the fresh milk or non-lactose milk or lactose-decomposed milk intended for persons having a predisposition to lactose intolerance. Further, the raw milk may include demineralized milk intended for patients on which intake limits to minerals, such as sodium, are imposed and calcium-enriched milk intended for patients who are recommended to take in a mineral such as calcium.

[0024]

The present antibacterial peptide-inducing agent has no particular limitation to the number of lactic acid bacteria, and it is essential only that lactic acid bacteria be incorporated into the agent. However, it is appropriate that the present antibacterial peptide-inducing agent contain on a per-pack basis one hundred million or more lactic acid bacteria, preferably five hundred million or more lactic acid bacteria, far preferably one billion or more lactic acid bacteria. It can be expected that the greater the number of lactic acid bacteria, the higher the effect of the invention will become. However, in consideration of the growth of lactic acid bacteria in general, the upper limit of the number of lactic acid bacteria is 10 trillion. Additionally, in the cases where both lactic acid bacteria of the genus *Lactobacillus* and lactic

acid bacteria of the genus *Streptococcus* are incorporated, it is appropriate that the number of lactic acid bacteria of the genus *Lactobacillus* be at least ten million and the number of lactic acid bacteria of the genus *Streptococcus* be at least one hundred million, it is preferred that the number of lactic acid bacteria of the genus *Lactobacillus* be at least fifty million and the number of lactic acid bacteria of the genus *Streptococcus* be at least five hundred million, it is more preferred that the number of lactic acid bacteria of the genus *Lactobacillus* be at least one hundred million and the number of lactic acid bacteria of the genus *Streptococcus* be at least one billion, and it is most preferred that the number of lactic acid bacteria of the genus *Lactobacillus* be at least five hundred million and the number of lactic acid bacteria of the genus *Streptococcus* be at least five billion. It can be expected that the greater the number of lactic acid bacteria of the genus *Lactobacillus* and the greater the number of lactic acid bacteria of the genus *Streptococcus*, the higher the effect of the invention will become. However, in consideration of the growth of lactic acid bacteria in general, the upper limit of the number of lactic acid bacteria is one trillion in the case of *Lactobacillus Bulgaricus* and 10 trillion in the case of *Streptococcus Thermophilus*. Further, in the case of containing e.g. both *Lactobacillus delbrueckii* subspecies *bulgaricus* and *Streptococcus salivarius* subspecies *thermophilus*, it is preferred that the number of *Lactobacillus delbrueckii* subspecies *bulgaricus* be at least ten million and the number of *Streptococcus salivarius* subspecies *thermophilus* be at least one hundred million, it is more preferred that the number of *Lactobacillus delbrueckii* subspecies *bulgaricus* be at least fifty million and the number of *Streptococcus salivarius* subspecies *thermophilus* be at least five hundred million, it is still more preferred that the number of *Lactobacillus delbrueckii* subspecies *bulgaricus* be at least one hundred million and the number of *Streptococcus salivarius* subspecies *thermophilus* be at least one billion, and it is most preferred that the number of *Lactobacillus delbrueckii* subspecies *bulgaricus* be at least five hundred million and the number of *Streptococcus*

salivarius subspecies thermophilus be at least five billion. It can be expected that the greater the number of Lactobacillus delbrueckii subspecies bulgaricus and the greater the number of Streptococcus salivarius subspecies thermophilus, the higher the effect of the invention will become. However, in consideration of the growth of lactic acid bacteria in general, the upper limit of the number of Lactobacillus delbrueckii subspecies bulgaricus is one trillion and the upper limit of the number of Streptococcus salivarius subspecies thermophiles is ten trillion.

[0025]

The present antibacterial peptide-inducing agent has no particular restriction as to the weight per unit pack, but as far as the per-gram number of lactic acid bacteria present in the antibacterial peptide-inducing agent is one hundred million or more, the per-pack weight of the antibacterial peptide-inducing agent is desirably from 10 g to 500 g, preferably from 25 g to 250 g, far preferably from 50 g to 200 g, optimally from 75 g to 150 g. The unit pack in the invention may have the form of not only a bag, a box or a case but also a small packet which is packed in such a receptacle and contains the each-time or daily dosage of the agent. It is possible to make a product in the form of a package containing the agent in total quantity appropriate to intake for several days, say a week, or a product containing a plurality of small packets in which the agent is individually wrapped.

[0026]

In one embodiment of the invention, for the purpose of enhancing the effect of promoting induction and secretion of antibacterial peptides, it is appropriate that the intake of lactic acid bacteria and/or cultures thereof as well as the antibacterial peptide-inducing agent containing them be continued for at least 3 weeks, preferably at least 5 weeks, far preferably at least 8 weeks.

[0027]

The lactic acid bacteria and cultures thereof can be taken in safely, and hence the upper limit to their intake duration is not particularly restricted, and the intake of them can be continued permanently. From the viewpoint of producing sufficient effect on induction of antibacterial peptides, estimated duration of the intake may be up to 8 weeks.

[0028]

The term milk as used in the invention is intended to include at least one of milk, dairy products and lactoprotein. Examples of milk usable in the invention include animal milk, such as cow's milk, ewe's milk and nanny goat's milk, processed goods of such milk, sterilized milk, defatted milk, whole fat milk powder, partially defatted powdered milk, whole fat concentrated milk, defatted concentrated milk, cream, buttermilk, whey, whey protein concentrate (WPC), whey protein isolate (WPI), α -lactoalbumin (α -La), β -lactoglobulin (β -Lg), α -casein, β -casein, κ -casein and non-protein nitrogen.

[0029]

The lactic acid bacteria according to the invention can also be used for medicines or food and drink. The medicines or food and drink containing the lactic acid bacteria according to the invention are useful in that they have the effect of promoting expression of antibacterial peptides, and can be used as medicines or food and drink for prevention or treatment of microbial infections. When the lactic acid bacteria according to the invention are used for medicines or food and drink, one strain thereof may be used alone, or two or more strains thereof may be used in combination.

[0030]

In using the lactic acid bacteria according to the invention for the medicines or food and drink, it doesn't matter what state they are in. Even when the lactic acid bacteria according to the invention are in any state, e.g. a state of viable bacteria, killed bacteria or treated bacteria, they can be used for the medicines or food and drink. The treated matter of

lactic acid bacteria according to the invention, which can be incorporated into the medicines or food and drink, may be culture or concentrate of the lactic acid bacteria, pasted matter obtained by working the lactic acid bacteria into paste, spray, freeze, vacuum or drum dried matter of the lactic acid bacteria, liquid obtained by dispersing the lactic acid bacteria into a medium, matter obtained by diluting the lactic acid bacteria with a diluent, pulverized matter obtained by pulverizing the dried matter with a mill, or the like.

[0031]

Further, food and drink according to the invention can also be applied to food with health claims and food for invalids. The system of food with health claims has been laid down for not only ordinary food but also tablets, capsules and the like on the basis of domestic and foreign trends and harmonization with the traditional system of food for specified health uses, and consists of two types, food for specified health uses (specially permitted type) and food with nutrient function claims (standardized type). By administering the lactic acid bacteria according to the invention into a human or animal body as food for special dietary uses including food for specified health uses, or food with nutrient function claims, prevention of various infections becomes possible.

[0032]

In an embodiment of the invention, it is appropriate that the present agent for increasing an antibody titer have an indication of descriptions concerning its uses, effects and functions, the kinds of active components therein, the kinds of functional components therein, the ways of taking in, and so on. The “indication” of the agent can bear descriptions suitable for each individual use, such as the use as a medicine, a quasi-medicine, health functional food, food for specified health uses, food with nutrient function claims, general food, health supplementary food, health food, a supplement, an enteral nutrient, oral cosmetics or feed.

The term “indication” used in the present description includes all of indications with

intention of making the foregoing descriptions known to customers, and more specifically, it includes all indications as far as they are indications capable of recalling or analogically inferring the foregoing descriptions indicated, irrespective of the indication purpose, indication contents, targets indicated and a medium used. As examples of a way to offer the indication of the foregoing descriptions, mention may be made of the way of indicating the foregoing descriptions on product packages or cases, the way of presenting or distributing product advertisements, price lists or trade documents bearing indications of the foregoing descriptions, and the way of electromagnetically providing information containing the foregoing descriptions (e.g. through Internet or the like).

[0033]

As an embodiment of the invention, it is appropriate that the indication of intended uses be affixed to packaged products prepared using a method relating to the invention by which expression of antibacterial peptides is augmented, secretion of the antibacterial peptides is promoted or infections are prevented or alleviated. For example, in the case of food and drink prepared using the method of augmenting expression of the antibacterial peptides, it is appropriate that packages of the food and drink having the effect of augmenting expression of antibacterial peptides have an indication of application to augment of antibacterial peptides, e.g. the wording “use for augment of antibacterial peptides”, while it is appropriate that packages of the food and drink containing the lactic acid bacteria have an indication of e.g. the wording “use for augment of antibacterial peptides” or the wording “use for prevention of infections”.

By the way, wordings used for providing the foregoing indications are not limited to the wording “use for augment of antibacterial peptides” and the wording “use for prevention of infections”, and it is needless to say that other wordings are included in the scope of the invention as far as they are wordings expressing the effect of activating energy metabolism.

As to such wordings, it is possible to adopt indications e.g. based on various uses which can be recognized by customers to be effective in augmenting antibacterial peptides and prevention of infections.

[0034]

When the lactic acid bacteria according to the invention are used in food and drink, the food and the drink have no particular restrictions as to the kinds thereof. Examples of food and drink into which the lactic acid bacteria can be processed include various kinds of foods and drinks (such as milk, processed milk, refreshing drinks, fermented milk, yogurt, cheese, other dairy products, bread, biscuits, crackers, pizza crust, formula milk, liquid diet, food for patients, powdered milk for infants or the like, powdered milk for pregnant women and nursing mothers, and nutritional foods). Such foods and drinks can be manufactured in accordance with manufacturing processes applicable to usual food compositions. For example, the lactic acid bacteria according to the invention or treated matter thereof may be used as they are, or they may be used as a mixture with another food or drink in its entirety or ingredients thereof. In addition, the forms of the food and the drink have no particular restrictions, and it doesn't matter specifically what forms the food and the drink have so long as they are those of usual food and drink. The food and drink may take any of forms including but not limiting to a solid form (including a powder form and a granule form,), a paste form, a liquid form and a suspension form. Among various processed foods and drinks, a milk-based drink, fermented milk, a refreshing drink, a jelly drink, a tablet and powdered food are preferable to others, and yogurt in particular is preferred over others.

[0035]

Into the food and drink containing the lactic acid bacteria according to the invention can be incorporated any of ingredients present in usual foods, such as water, protein, glucide, lipid, vitamins, minerals, organic acids, fruit juice, flavor, functional ingredients and food

additives. Examples of a protein source usable in manufacturing such food and drink include proteins and protein-containing raw materials used generally in manufacturing foods, specifically flora and fauna proteins such as whole fat powdered milk, defatted powdered milk, partially defatted powdered milk, casein, whey powder, whey protein, whey protein concentrate, whey protein isolate, α -casein, β -casein, κ -casein, β -lactoglobulin, α -lactoalbumin, lactoferrin, soy bean protein, egg protein and meat protein, and hydrolysis products of the proteins recited above. Examples of a glucide source include processed starches (such as dextrin and others including soluble starch, British starch, oxidized starch, starch ester, starch ether and so on) and dietary fiber. Examples of a lipid source include animal oils and fats, such as lard, fish oil, and fractionated, hydrogenated, or ester exchanged oils of those oils; and vegetable oil and fats, such as palm oil, safflower oil, corn oil, rape oil, coconut oil, and fractionated, hydrogenated or ester exchanged oils of those oils. Examples of vitamins include vitamin A, carotenes, vitamin B group, vitamin C, vitamin D group, vitamin E, vitamin K group, vitamin P, vitamin Q, Niacin, nicotinic acid, pantothenic acid, biotin, inositol, choline and folic acid, and examples of minerals include calcium, potassium, magnesium, sodium, copper, iron, manganese, zinc and selenium. Examples of an organic acid include malic acid, citric acid, lactic acid and tartaric acid. Examples of a functional ingredient include oligosaccharide, glucosamine, collagen, ceramide, royal jelly and polyphenols. Examples of food additives include an emulsifier, a stabilizer, a thickener, a gelling agent, a sweetener, a sour agent, a preservative, an antioxidant, a pH adjuster and a coloring agent. Various ingredients derived from milk, such as butter, lactoserum minerals, cream, whey, non-protein nitrogen, sialic acid, phospholipid and lactose, are examples of ingredients which can be utilized suitably in manufacturing food and drink containing lactic acid bacteria according to the invention.

Two or more of those ingredients can be used in combination. The raw materials of

those ingredients may be any of natural substances, processed goods of natural substances, synthetic products and/or foods containing them in quantities.

[0036]

The lactic acid bacteria according to the invention can be made into medicines for prevention or treatment of infections. In manufacturing such medicines, the *Lactobacillus* *bijidus* or lactic acid bacteria according to the invention can be used in the form of viable cells or processed cells which have undergone sterilizing and then grinding or no grinding. Additionally, the bacteria used may be a single strain or a mixture of two or more strains.

[0037]

The proportion of the lactic acid bacteria according to the invention in such a medicine as mentioned above can be decided as appropriate according to application purpose and uses (e.g. as a preventive or a remedy). As an example, the proportion of the lactic acid bacteria to the whole medicine can be chosen from a range of 0.001 to 100 % (w/w), notably a range of 0.1 to 100%, but the invention should not be construed as being limited to such a range.

[0038]

The dose of the medicine containing as an active ingredient the lactic acid bacteria according to the invention can be chosen as appropriate in consideration of various factors, such as an administration route, an age, weight and symptoms of an animal including a human being as the target for administration. As an example of an appropriate dose, mention may be made of 1 to 100 mg/kg/day in terms of the active ingredient, but the invention is not limited to such a dose. For example, in the case of taking the medicine over the long term for the preventive purpose, the dosage may be below the foregoing range. Alternatively, because there is no safety problem to find in the active ingredient of the invention, it is thought that there's nothing wrong with taking the medicine in a dosage beyond the foregoing

range.

[0039]

For the purpose of making the lactic acid bacteria according to the invention reach to the interior of intestines, it is appropriate for the dosage form of the medicine to be a dosage form allowing oral administration. Examples of an appropriate dosage form of the medicine according to the invention include the forms of a tablet, a coated tablet, a capsule, granules, powder, liquid, syrup and a troche. These various formulations can be prepared through the incorporation of auxiliary agents commonly used in the art of medicinal preparation, such as an excipient, a binder, a disintegrant, a lubricant, a colorant, a flavoring agent, a dissolution assistant, a suspending agent and a coating agent, into the bacterial cells and/or treated matter thereof as the main medicine according to the invention.

[0040]

When the present antibacterial peptide-inducing agent is used as a medicine and subjected e.g. to oral administration, it is possible to administer (take) the lactic acid bacteria or a fermented material thereof as it is, but the present agent may be used e.g. in the form of tablets, granules, pulverized particles, capsules or powders which are formulated in accordance with a general method for production of medicines.

[0041]

The present antibacterial peptide-inducing agent holds the promise of producing its effects on all areas of the alimentary canal, and the term “the alimentary canal” used herein refers to a series of organs including the oral cavity, the pharynx, the gullet, the stomach, the duodenum, the small intestine, the large intestine, the appendix and the anus. Herein, names of representative regions of the alimentary canal are enumerated, but the administration (taking) of the present antibacterial peptide-inducing agent holds the promise of producing similar effects also on specific regions of the alimentary canal, such as the colon (the lower

portion of the small intestine), the ileum, the jejunum (the upper portion of the small intestine), the upper abdominal alimentary canal, the lower abdominal alimentary canal, the upper region of large intestine and the lower region of large intestine, as far as the names of these regions are well-known and included in the category of the alimentary canal in the technical common sense.

[0042]

The present antibacterial peptide-inducing agent has its effects on all of mammals including human being, and hence the taking (administration) of this antibacterial peptide-inducing agent can also be applied to treatment and/or preventive for various diseases. Those which fits into the term mammals used herein are all of beings classified as mammals, such as well-known livestock, pets, appreciation animals and wild animals, with examples including human beings, cattle, pigs, sheep, dolphins, whales, tigers, lions, cheetahs, hippopotami, giraffes, camels, alpacas, dogs, cats, monkeys, foxes, raccoon dogs, bears, squirrels, seals, sea lions, pandas, wild boars, deer, horses, orangutans and kangaroos. On the other hand, it is also possible to apply the administration and/or taking of the present antibacterial peptide-inducing agent to alimentary canal-possessing animals other than mammals, such as insects, reptiles, birds, fish, amphibians and mollusks. The term various diseases used herein has no particular restrictions so long as the diseases can be treated and/or prevented through the induction of antibacterial peptides, and it is intended to include infectious diseases caused by opportunistic pathogens, infectious diseases caused by pathogenic bacteria and fungi, *Helicobacter pylori* infection, bacteria translocation, persistent inflammation of intestinal mucosa, change in balance of intestinal flora (e.g. collapse in balance of intestinal flora), diseases induced by these inflammations, Crohn's disease, inflammatory colitis such as ulcerative colitis, and excess inflammation and allergic symptoms resulting from induction of regulatory T cell differentiation.

Examples

[0043]

The invention will now be illustrated in more detail by reference to the following examples, but the invention should not be construed as being limited to these examples in any way.

[0044]

[Test Example 1]

(Laboratory Animal)

Old-age mice were examined for influence of intake of lactic acid bacteria upon induction of antibacterial peptides. ICR male mice of age 8 months (Japan SLC, Inc.) were received as a group of created old-age mice, and acclimatized for one week. During this period, refuges made of paper were placed in a cage for the purpose of protecting the mice against fighting and stress. After the acclimatization, venous blood was taken from the tail of each of the mice, and therefrom plasma was extracted by centrifugal separation. On the plasma obtained, concentrations of blood plasma IL-1 β , blood plasma IL-6 and blood plasma TNF α as typical cytokine increasing in concentration at the time of infection were measured individually by the ELISA method. The old-age mice were separated into two groups (n=39 in the two groups) so that the two groups became equal in weight and concentration of each cytokine. As to the control group, ICR male mice of age 3 months were used as young-age mice.

[0045]

(Milk-Fermented Material)

Raw milk, a dairy product and raw water were compounded in amounts that the resulting composition had a milk fat content of 3.0 weight% and a nonfat milk solid content of 9.5 weight%, heated up to a temperature around 70°C, subjected to homogenization

treatment under a pressure of 16 MPa to make the diameters of milk fat globules uniform, and then sterilized under 2 minutes' heating at 95°C. After the heat sterilization, the composition was cooled to a temperature between 40°C and 45°C, admixed with a mixed starter consisting of *Lactobacillus Bulgaricus* and *Streptococcus Thermophilus* isolated from commercially available "Meiji Bulgaria Yogurt", made to ferment at a temperature between 40°C and 45°C for 4 hours, adjusted to have a lactic acid acidity of 0.9 weight%, and then cooled to a temperature of 10°C or below. The thus processed composition was described as a lactic acid bacteria fermentation material of milk, and adopted as a test sample. In 1 g of the test sample were present one hundred million of *Lactobacillus delbrueckii* subspecies *Bulgaricus* and one billion of *Streptococcus salivarius* subspecies *Thermophilus*.

[0046]

(Method for Administering Test Sample to Laboratory Animal)

The test sample was administered to the mice as follows. A basic feed AIN-93M (containing 14 weight% of casein, 0.18 weight% of L-cystine, 46.5692 weight% of corn starch, 15.5 weight% of pregelatinized corn starch, 10 weight% of sucrose, 4 weight% of soybean oil, 5 weight% of cellulose powder, 3.5 weight% of AIN-93 mineral mixture, 1 weight% of AIN-93 vitamin mixture, 0.25 weight% of choline bitartrate and 0.0008 weight% of tert-butylhydroquinone) was kneaded with the test sample and water, and rolled up into balls, and then these balls were administered to the mice. The test sample-incorporated feed was administered to one of the old-age mice creation groups, and this group was referred to as a test-sample old-age mice creation group (test sample-administered group) for the sake of convenience. In addition, the test sample-free basic feed was administered to the other of the old-age mice creation groups, and this group was referred to as a basic-feed old-age mice creation group (negative group). Further, the test sample-free basic feed was administered to the young-age mice control group, and this group was referred to as a basic-feed young-age

mice control group (basic-feed young-age group). The administration period was 20 months in the cases of the test sample-administered group and the negative groups, while it was 2 months in the case of the basic-feed young-age group. The dose of each feed was set at 20 g/kg(body weight)/day. During the administration period, body-weight and intake measurements were made at regular time intervals, and further 150 μ L of blood was taken once every two months, and therefrom blood plasma was extracted. After the expiration of the administration period, abdominal operations were performed and the jejunum, the ileum and the colon were removed, and then portions of these tissues were treated at 4°C for one night with a 10-fold volume of RNA later solution (a product of Ambion Inc.) as tissue samples for RNA extraction, and then preserved at -80°C. The remainder tissues were flash-frozen in liquid nitrogen, and then preserved at -80°C.

[0047]

(DNA Microarray Analysis)

The foregoing tissues obtained from each group were subjected to RNA extraction. The extraction was carried out by means of an RNeasy kit (a product of QIAGEN). Total RNA derived from the mice in the same group were mixed together in equal amounts to prepare a sample for DNA microarray analysis. In the concrete preparation method, a reagent recommended by Affymetrix was used. By means of a 3' IVT' Expression kit, each of total RNA samples synthesized cDNA with the aid of a primer containing T7 promoter sequence, and cRNA was synthesized by using the cDNA as a template in the presence of T7 RNA polymerase and at the same time labeled with biotin. The biotin-labeled cRNA was hybridized to Mouse Genome 430 2.0 Array (a product of Affymetrix). This hybridization was performed at 45°C for 16 hours by means of an oven for hybridization use (GeneChip™ Hybridization Oven 630, a product of Affymetrix). Further, the array was cleaned by means of an automatically cleaning and dyeing apparatus (GeneChip™ Fluidics Station 450, a

product of Affymetrix), and thereafter the single stranded cRNA bound on the array was given florescent labeling with Streptavidin-phycoerythrin. By the way, the automatically cleaning and dyeing apparatus was controlled by means of machine control software (Affymetrix™ GeneChip™ Command Console™ Software Ver. 2 (hereafter abbreviated as AGCC), a product of Affymetrix).

[0048]

Image data was scanned off the array having undergone the fluorescent labeling operation by means of a laser scanner (GeneChip™ Scanner 30007G, a product of Affymetrix). Additionally, capture of the image data by means of the scanner was made by the use of the AGCC software. The extracted image data was standardized by the use of analysis software (Expression Console™ ver. 1.1, a product of Affymetrix), and further a comparative analysis concerning gene expression levels of different groups was made by the use of GeneSpring GX analysis software (GeneSpring GX ver. 11, a product of Agilent Technologies) or GCOS (a product of Affymetrix). The thus obtained gene expression levels of the test sample-administered group and the negative group were worked out as relative values, with the gene expression level of the basic-feed young-age group being taken as 1.0

[0049]

DNA microarray analyses were done on each group's jejunum and colon samples, respectively, and antibacterial peptide genes affected by ageing or administration of a test sample were extracted.

[0050]

As to the jejunum, the expression levels of RegIII α and RegIII γ genes belonging to the Reg peptide family as one species of antibacterial peptides were reduced in the negative group, and the expression levels of Defensin rs and Defensin rs7 genes belonging to the Defensin family as another species of antibacterial peptides were reduced remarkably in the

negative group. The expression levels of the 4 species of genes in the test sample-administered group were two or more times as high as those in the negative group and approximated to those in the basic-feed young-age groups. In addition, the expression level of RegII gene in the jejunum was high in the negative group as compared with that in the basic-feed young-age group, and there was a trend to increase markedly the expression level of RegII gene in the test sample-administered group (see Table 1).

[0051]

As to the ileum, the expression level of Defensin β I belonging to the Defensin family as one species of antibacterial peptides was reduced in the negative group. The increasing tendency of the expression level of this gene was stronger in the test sample-administered group than in the negative group and the basic-feed young-age group (see Table 2).

[0052]

As to the colon, the relative expression levels of RegI gene and RegIII γ belonging to the Reg peptide family as one species of antibacterial peptides were markedly reduced in the negative group. The increasing tendency of the relative expression levels of these two genes was strong in the test sample-administered group as compared with those in the negative group (see Table 3).

From these experimental results, it can be said that the expression of antibacterial peptide genes, except for RegII, in the small intestine (alimentary canal) was reduced by ageing and intake of a sample containing the lactic acid bacteria according to the invention made it possible to prevent the expression of antibacterial peptide genes from being reduced by ageing.

[0053]

<Table 1>

Expression Levels of Antibacterial Peptide Genes in Jejunum

Gene	Test Sample-Administered Group	Negative Group
RegII	27.9	2.83
RegIII α	0.2	0.1
RegIII γ	0.8	0.35
Defensin-rs	0.4	0.07
Defensin-rs7	0.3	0.01

[0054]

<Table 2>

Expression Level of Antibacterial Peptide Gene in Ileum

Gene	Test Sample-Administered Group	Negative Group
Defensin β I	1.87	0.87

[0055]

<Table 3>

Expression Levels of Antibacterial Peptide Genes in Colon

Gene	Test Sample-Administered Group	Negative Group
RegI	1	0.47
RegIII γ	0.5	0.03

[0056]

[Test Example 2]

(Quantitative PCR Analysis)

By means of a transcription kit for real time RT-PCR, Takara Prime Script RTMasterMix (a product of Takara Bio Inc.), cDNA was synthesized from the RNA sample extracted from the foregoing tissues in each group. By using the synthesized cDNA as a template and a real time PCR reagent, SYBR Premix Ex TaqII (Tli RNaseH Plus) (a product of Takara Bio Inc.), quantitative PCR reaction was conducted and detected with a thermal cycler, Thermal Cycler Dice Real Time System (a product of Takara Bio Inc.). In addition, for the purpose of determining quantitatively the expression levels of RegIII β and RegIII γ ,

this time the expression levels of these genes were worked out as relative expression levels by performing relative quantification utilizing a housekeeping gene Actb as endogenous control. Incidentally, the statistical processing method adopted herein was Mann-Whitney U-test or G-test. The cases in which the p values were below 0.05 were assessed as having a significant difference.

Comparisons on the relative expression levels of RegIII β and RegIII γ were made between the test sample-administered group, the negative group and the basic-feed young-age group. As to the negative group, the relative expression levels of RegIII β and RegIII γ were significantly low as compared to those in the basic-feed young-age group. In contrast, there were no significant differences between the test sample-administered group and the basic-feed young-age group, and these groups were equal in the relative expression levels of RegIII β and RegIII γ .

Industrial Applicability

[0057]

The invention makes it possible to provide an inducing agent by which internal secretion of antibacterial peptides exerting the biological self-defense mechanism possessed by human beings is induced in safety without harmful aftereffects. Further, intake of the present inducing agent for internal secretion of antibacterial peptides induces the antibacterial peptides in vivo, and thereby immunity enhancement ascribable to protection against various infections, antiaging, extension of healthy life expectancy and so on can be expected.

Claims

[Claim 1]

An antibacterial peptide-inducing agent, characterized by containing lactic acid bacteria or a material fermented with lactic acid bacteria or both as active ingredients and inducing gene expression of antibacterial peptides.

[Claim 2]

The antibacterial peptide-inducing agent as described in claim 1, wherein the antibacterial peptides are either Defensins or Regenerating Islet-derived family of peptides (Reg peptides), or both.

[Claim 3]

The antibacterial peptide-inducing agent as described in claim 1 or 2, wherein the lactic acid bacteria are either lactic acid bacteria of the genus *Lactobacillus* or lactic acid bacteria of the genus *Streptococcus*, or both.

[Claim 4]

An antibacterial peptide-inducing composition containing the antibacterial peptide-inducing agent as described in any of claims 1 to 3.

[Claim 5]

The antibacterial peptide-inducing composition as described in claim 4, wherein the lactic acid bacteria are a combination of one or more than one strain selected from lactic acid bacteria of the genus *Lactobacillus* and one or more than one strain selected from lactic acid bacteria of the genus *Streptococcus*.

[Claim 6]

The antibacterial peptide-inducing composition as described in claim 4, which contains at least one hundred million of lactic acid bacteria on a per-pack basis.

[Claim 7]

The antibacterial peptide-inducing composition as described in any one of claims 1 to 6, wherein the lactic acid bacteria are viable bacteria.

[Claim 8]

The antibacterial peptide-inducing composition as described in any one of claims 4 to 7, which is a food or medicine composition inducing gene expression of antibacterial peptides and having an infection prophylactic action.

[Claim 9]

The food or medicine composition having an infection prophylactic action as described in claim 8, which is taken in orally or enterally to induce antibacterial peptides.

[Claim 10]

Use of lactic acid bacteria or a material obtained through fermentation with the lactic acid bacteria or a mixture thereof as active ingredients for gene expression of antibacterial peptides in manufacturing an antibacterial peptide-inducing agent.

[Claim 11]

Use of lactic acid bacteria or a material obtained through fermentation with the lactic acid bacteria or a mixture thereof as active ingredients for gene expression of antibacterial peptides in manufacturing an antibacterial peptide-inducing composition containing an antibacterial peptide-inducing agent.

[Claim 12]

Use of lactic acid bacteria or a material obtained through fermentation with the lactic acid bacteria or a mixture thereof as active ingredients for gene expression of antibacterial peptides in manufacturing a food or medicine composition containing an antibacterial peptide-inducing agent and having an infection prophylactic action.