

Specification

Title of Invention

PROPHYLACTIC AGENT FOR PNEUMOCOCCAL INFECTION

Technical Field

[0001]

The present invention provides a prophylactic agent for pneumococcal infection, i.e. a composition for preventing pneumococcal infection and preventing or inhibiting the infection from becoming severe during the occurrence of the infection.

Background Art

[0002]

Chain coccus causing pneumonia, *Streptococcus pneumoniae* is also called pneumococcus, and its infection causes respiratory tract infectious diseases such as pneumonia and systemic infectious diseases. Particularly in elderlies, infants and the like, pneumococcal infection frequently leads death in many cases. Note that, in the pneumococcal infection, a penicillin antibiotic is administered. In addition, a vaccine is generally administered in preventing pneumococcal infection.

[0003]

However, in administration of an antibiotic, no sufficient therapeutic effects may be expected due to emergence of resistant bacteria. Also, vaccine administration has disadvantages of possibility of side effects and high cost. Consequently, safe and inexpensive means for preventing pneumococcal infection and preventing or inhibiting the infection from becoming severe during the occurrence of the pneumococcal infection are desired.

[0004]

On the other hand, there have been recently many studies in which physiological activities in microorganisms are focused on in light of utilization of the probiotics. Among them, lactic acid bacteria which are used for manufacturing yoghurt, cheese and the like have various physiologically active actions, and thus it is considered that their utility values are high. For example, *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1 is known for extracellular production of acidic polysaccharides, and it has an effect to prevent autoimmune diseases (Patent Document 1) and an action to improve skin (Patent Document 2). In addition, the polysaccharide produced by the lactic acid bacteria has an action to activate an NK cell (Patent Document 3) and an antiviral action (Patent Document 4), and contributions to improvement of skin fitness and texture, anticancer therapy, and prevention and treatment for virus infection are expected.

[0005]

Furthermore, there have been reports that lactic acid bacteria other than the above-mentioned lactic acid bacteria also have various physiological activities, and for example, there is a report that killed bacterial bodies of *Lactobacillus pentosus* strain b240 significantly decrease the number of intrapulmonary viable bacteria in pneumococcal pneumonia (Non Patent Document 1). However, there is no report that a lactic acid bacterium other than this B240 strain prevents pneumococcal infection and contributes to preventing the infection from becoming severe during the occurrence of the infection.

Prior art documents

Patent Document

[0006]

Patent Document 1: JP 2000-247895 A

Patent Document 2: JP 2007-039423 A

Patent Document 3: JP 2005-194259 A

Patent Document 4: WO 2011/65300

Non Patent Document

[0007]

Non Patent Document 1: A. Tanaka et al., The Society for Applied Microbiology, 2011, 35-43

Summary of Invention

Technical Problem

[0008]

The present invention was made in consideration of the above-described background art, and its purpose is to provide a food composition or a pharmaceutical composition which prevents or inhibits pneumococcal infection and the infection from becoming severe during the occurrence of the infection by routine ingestion.

Solution to Problem

[0009]

The prophylactic agent for pneumococcal infection according to the present invention is characterized in that it comprises a fermentation product of *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1.

Advantageous Effects of Invention

[0010]

Ingestion of the prophylactic agent according to the present invention prevents pneumococcal infection, and prevents or inhibits the infection from becoming severe during the occurrence of the infection.

Brief Description of Drawings

[0011]

Fig. 1 is a figure showing comparison of survival rates after infection.

Fig. 2 is a figure showing comparison of the number of the intrapulmonary viable bacteria.

Fig. 3 is a figure showing comparison of the number of the viable bacteria fixed on upper respiratory tracts.

Description of Embodiments

[0012]

The prophylactic agent for pneumococcal infection according to the present invention comprises a fermentation product of *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1 (FERM BP-10741: hereinafter referred to "Lactobacillus bulgaricus R-1 strain") as an active ingredient. The *Lactobacillus bulgaricus* R-1 strain is known, and is originally deposited at International Patent Organism Depositary Center of National Institute of Technology and Evaluation (IPOD, NITE) (Central 6, 1-1-1, Higashi, Tsukuba-city, Ibaraki-Pref., 305-8566, Japan) with accession number IPOD FERM BP-10741 (Date of the deposit: February 22, 1999).

[0013]

The fermentation product is obtained by fermenting (culturing) various bases using the *Lactobacillus bulgaricus* R-1 strain. In the present invention, both of fermentation products from which viable bacteria and killed bacteria were removed and fermentation products containing viable bacteria and killed bacteria (from which viable bacteria and killed bacteria were not removed) can be used, but the fermentation products containing viable bacteria are preferably used in light of probiotics.

[0014]

For the base to be used for fermentation, any base will do as long as it can form an environment in which fermentation may occur as a result of growth or proliferation of the *Lactobacillus bulgaricus* R-1 strain. The base may be a food material e.g. milk of humans or animals, a vegetable, a fruit, a bean and a grain, and may also be a culture medium or raw material milk for growth or proliferation of microorganisms. The base is preferably a food material which can be ingested as a food after fermentation, specifically a culture medium or a raw material milk containing raw milk (unpasteurized

milk), pasteurized milk, whole concentrated milk, whole milk powder, skim milk powder, defatted concentrated milk, milk protein concentrate (MPC), whey, whey powder, demineralized whey, demineralized whey powder, whey protein concentrate (WPC), whey protein isolate (WPI), α -lactalbumin, β -lactoglobulin, casein, sodium caseinate, calcium caseinate, cream, butter, soy milk, etc., and may be a culture medium or raw material milk with these food materials containing carbohydrates (including lactose), minerals, vitamins and yeast extracts.

[0015]

Additionally, lactic acid bacteria other than *Lactobacillus bulgaricus* R-1 strain and/or other various fermentative bacteria such as *Bacillus natto* and yeast can be concomitantly used in fermentation, specifically *Streptococcus thermophilus* which is used as a starter bacterium in manufacture of yoghurt and *Bacillus natto* which is used for fermentation of natto can be used. The *Streptococcus thermophilus* to be concomitantly used is exemplified by a *Streptococcus thermophilus* OLS3059 strain. The *Streptococcus thermophilus* OLS3059 strain is originally deposited at International Patent Organism Depositary Center of National Institute of Technology and Evaluation (IPOD, NITE) (Central 6, 1-1-1, Higashi, Tsukuba-city, Ibaraki-Pref., 305-8566, Japan) with accession number IPOD FERM BP-10740 (Date of the deposit: February 22, 1999).

[0016]

Method and conditions for fermentation (culture) can be optionally selected by those skilled in the art and arbitrarily determined according to the actually selected base. Method and conditions for fermentation (culture) cannot be particularly limited as long as the conditions and method allow growth or proliferation of the *Lactobacillus bulgaricus* R-1 strain. The temperature for fermentation (culture) is e.g. room temperature (20 to 30°C) to 45°C, preferably 35 to 45°C, more preferably 40 to 45°C, and the time for fermentation is e.g. 1 to 72 hours, preferably 2 to 48 hours, more preferably 4 to 16 hours.

[0017]

The prophylactic agent for pneumococcal infection according to the present invention comprises fermentation products and may be both a composition prepared by adding other materials and additives to the fermentation product and a fermentation product itself. In this case, the fermentation product may be e.g. a fermented milk, a lactic acid bacterium beverage and a natural cheese comprising milk (of humans or animals) as a raw material. In addition, the fermented milk means a product defined by Ministerial Ordinance concerning Milk, specifically a product which was prepared by steps that a milk such as raw milk, cow's milk, special milk, raw goat milk, pasteurized goat milk, raw sheep milk, homogenized milk, low-fat milk, non-fat milk and processed milk, or a milk containing non-fat milk solids equal to or surpassing the above-mentioned milk, or the like was fermented with lactic acid bacteria or the like and then processed into a solid form, a paste form or a liquid form, or frozen. In addition, the fermentation product may be e.g. kimchi, tsukemono and pickles which are made of vegetables. Furthermore, it may be a polysaccharide-containing composition prepared by removing a liquid content from a fermentation product (culture solution) obtained using a culture medium for growth or proliferation of microorganisms by concentration, centrifugation, etc. Among them, the fermented milk containing a large number of viable bacteria is preferred.

[0018]

The prophylactic agent for pneumococcal infection according to the present invention is a pharmaceutical composition, and may also be food compositions such as food for specified health uses, functional food, health food, health supplemental food and supplement, or various compositions such as feed and cosmetic (oral cosmetic, etc.). In this case, for preparation of these compositions and the like, both fermentation products processed with concentration such as freeze-drying and solid-liquid separation and fermentation products themselves without such a processing can be used.

Although additives can be selected by those skilled in the art depending on the desired form and dosage form, they may be e.g. known additives used in formulation (productization) of pharmaceuticals or foods such as excipient like lactose and corn starch, binder, disintegrating agent, lubricant, flavoring agent, solubilizer, suspending agent and coating agent, bases such as water, oil, milk, vegetable, fruit juice, bean and grain, emulsifiers, stabilizers, thickeners, gelling agents, sweeteners, acidulants, preservatives, antioxidants, pH adjusters, flavors, and colorants.

[0019]

The prophylactic agent for pneumococcal infection according to the present invention has, as its form or dosage form, a form or a dosage form which allows humans or other animals to ingest it orally or parenterally. The form and the dosage form may be e.g. a tablet, a granule, a powder, a capsule, a liquid, an injection, a jelly and a gel. The food compositions also include so-called foods, and the foods are e.g. instant foods such as instant noodle, retort food, can, microwave food, instant soup/miso soup and freeze-dried food; beverages such as soft drink, fruit juice drink, vegetable drink, soy milk beverage, coffee beverage, tea beverage, powdered drink, concentrated drink and alcoholic beverage; wheat flour products such as bread, pasta, noodle, cake mix and bread crumbs; confectioneries such as candy, caramel, chewing gum, chocolate, cookie, biscuit, cake, pie, snack confectionery, cracker, Japanese confectionery and dessert confectionery; seasonings such as source, processed tomato seasoning, flavor seasoning, cooking mix, mop sauce, dressing, soup and mix for curry/stew; oils and fats such as processed oil and fat, butter, margarine and mayonnaise; dairy products such as milk beverage, fermented milk (such as yoghurt), lactic acid bacterium beverage, solid milk, natural cheese, processed cheese, ice cream and cream; processed agricultural products such as agricultural can, jam/marmalade and serial; frozen foods, liquid foods and enteral nutrients. The preferable aspect of the present invention is yoghurt such as plain yoghurt, hard yoghurt, soft yoghurt, drink type yoghurt in light of flavor.

[0020]

The prophylactic agent for pneumococcal infection according to the present invention has effects to prevent or inhibit the infection from becoming severe during the occurrence of the infection e.g. an effect to decrease a mortality rate after infection. Also, the prophylactic agent for pneumococcal infection according to the present invention has effects to inhibit fixation of pneumococcus and to prevent infection on not only a lower respiratory tract such as bronchus and lung but also an upper respiratory tract such as throat, pharynx and nasal cavity. Thus, not only patients with pneumococcus but also healthy persons who are non-affected individuals are the subjects for ingestion. In addition, the subjects for ingestion are mammals including humans, preferably humans. In this case, a daily intake of the prophylactic agent varies depending on age, gender and whether being affected or healthy, but it may be arbitrarily determined by a person skilled in the art. A lower limit of the daily intake is not less than 10^6 cfu, preferably not less than 10^7 cfu, more preferably not less than 10^8 cfu in terms of the *Lactobacillus bulgaricus* R-1 strain. Further, when the lower limit of the daily intake is grasped in terms of the extracellular polysaccharide, it is 100 μ g, preferably 500 μ g, more preferably 1 mg in terms of the extracellular polysaccharide. In addition, each form or dosage form mentioned above can be prepared so that it contains the daily intake of the bacteria and the extracellular polysaccharides. In this case, each form or dosage form mentioned above is prepared so that the daily intake for each form or dosage form is 5 - 1000 g (5 to 1000 ml), more preferably 50 - 500 g (50 - 500 ml), more preferably 100 - 200 g (100 - 200 ml). It is desirable that the fermentation product of the *Lactobacillus bulgaricus* R-1 strain is continuously ingested, and it prevents pneumonia and upper respiratory infection accompanied with pneumococcal infection and prevents or inhibits the infection from becoming severe during the occurrence of the infection by ingestion for preferably not less than one week, more preferably not less than two weeks, further preferably not less than three weeks.

[0021]

The prophylactic agent for pneumococcal infection according to the present invention is preferably attached with an indication that it prevents pneumococcal infection and prevents or inhibits the infection from becoming severe during the occurrence of the infection. The indication about prevention of pneumococcal infection as well as prevention and inhibition of increase of severity includes not only words "for prevention of pneumococcal infection" and "for inhibition of the infection from becoming severe during the occurrence of the infection " but also various indications allowing the subjects to recognize the effects of the prophylactic agent for infection according to the invention of this patent application, e.g. an indication permitted on the basis of the legal systems of various countries such as a system about food for specified health use based on Pharmaceutical Affairs Act and Health Promotion Act in Japan. Particularly an indication for healthcare application and an indication similar thereto are listed as typical examples. In addition, the indication means may include not only descriptions on a container packaging a fermentation product, a manual and an accompanying document, and advertisement goods such as flyer and newspaper but also announcements by images and sounds.

[0022]

The present invention provides a method for preventing pneumococcal infection including a step of ingesting or making a subject ingest the fermentation product of *Lactobacillus bulgaricus* R-1 strain, and/or a method for preventing or inhibiting the infection from becoming severe during the occurrence of the pneumococcal infection. Herein, the method for preventing pneumococcal infection and/or the method for preventing/inhibiting the infection from becoming severe during the occurrence of the pneumococcal infection include medical practices and non-medical practices, but the non-medical practices are preferable. Furthermore, "making a subject ingest" implies not only "to directly make a subject ingest" but also "to indirectly make a subject ingest

" by indicating a visual and/or audible specific purpose and effects i.e. by indicating that the fermentation product prevents pneumococcal infection and/or prevents or inhibits the infection from becoming severe during the occurrence of the pneumococcal infection. Indirectly making a subject ingest may include actions of a manufacturer on a dealer or a consumer, or of a dealer on a consumer, to propose, suggest and indicate to make a subject ingest the product by means of the indication that pneumococcal infection is prevented and that increase of severity during the occurrence of the pneumococcal infection is prevented or inhibited.

[0023]

Hereinafter, the present invention will be explained on the basis of Examples. It is indisputable that the present invention is not limited to the following Examples.

Examples

Example 1

[0024] [Preparation of fermentation product]

The *Lactobacillus bulgaricus* R-1 strain as a starter bacterium was added to a solution prepared so as to comprise a skim milk powder (fermented milk Mix) as the main component, which was fermented at 37°C for 16 hours. An amount of extracellular polysaccharides in this fermentation product (fermented milk) was measured to calculate a dose to a mouse. In the method for measuring the amount of the extracellular polysaccharides, proteins were removed from the fermentation product by trichloroacetic acid, and the polysaccharides (extracellular polysaccharides) were precipitated by ethanol precipitation and purified. Then, an amount of the precipitated polysaccharides (extracellular polysaccharides) was measured by HPLC of the gel filtration mode. In addition, this fermentation product (fermented milk) contained 725 µg/g of extracellular polysaccharides and 1.7×10^9 CFU/g of lactic acid bacteria.

[0025] [Verification of lung infection model (1)]

(Infection in mouse lung)

In C57BL/6 mice (female, 8 weeks old, n=9 or 10), a fermented milk-administrated group was orally administrated (once/day, for 21 days before administration, and after administration) with the fermentation product of the *Lactobacillus bulgaricus* R-1 strain (50 µg/mouse in terms of the amount of extracellular polysaccharide) everyday over the test period. Note that a control group was orally inoculated with a solution prepared so as to comprise skim milk powder (fermented milk Mix) as the main component. On the 22nd day from the start of the oral administration, 50 µl of suspension of the pneumococcus (*Streptococcus pneumoniae*) D39 strain was transnasally administered (1×10^8 CFU/mouse). After the inoculation of pneumococcus, the survival rate and the number of viable bacteria in lungs were compared between the groups.

[0026]

Herein, the number of the viable bacteria in the lungs was measured as below. Two days after the inoculation of pneumococcus, the mice were sacrificed, and the lungs were removed. The obtained lungs were aseptically segmentalized and suspended in physiological saline (1 ml). This suspension of the lung was homogenized by Shake Master NEO (Bio Medical Science, Tokyo, Japan). The obtained homogenate solution of the lung was applied to a blood agar medium (Becton, Dickinson and Company) and cultured at 37°C for 20 hours. After the culture, the number of the viable bacteria in the lung was measured by counting the grown colonies.

[0027]

The comparison of the survival rates is shown in Fig. 1, and the comparison of the number of the viable bacteria in the lung is shown in Fig. 2. In the control group, some mice died 4 days after the start of the oral administration, meanwhile in the fermented milk-administrated group, no mice died even 7 days after the start of the oral administration. That is, in the fermented milk-administrated group, the mortality rate is considerably decreased compared to the control group, and development of the

pneumonia accompanied with pneumococcal infection was inhibited. In addition, in the fermented milk-administrated group, the number of the infected cells out of the number of the viable bacteria in the lung was significantly low compared to the control group.

Example 2

[0028] [Verification of lung infection model (2)]

(Infection in mouse upper respiratory tract)

In C57BL/6 mice (female, 8 weeks old, n=9 or 10), a fermented milk-administrated group was orally administrated (once/day, for 21 days before administration, and after administration) with the fermentation product of the *Lactobacillus bulgaricus* R-1 strain (50 µg/mouse in terms of the amount of extracellular polysaccharide) everyday over the test period. Note that a control group was orally administrated a solution prepared so as to comprise skim milk powder (fermented milk Mix) as the main component. On the 22nd day from the start of the oral administration, 50 µl of suspension of the pneumococcus (*Streptococcus pneumoniae*) 1121 strain was transnasally inoculated (1×10^7 CFU/mouse). After the inoculation of pneumococcus, the number of the fixed bacteria in the upper respiratory tract (the number of the pneumococcus which were fixed on the upper respiratory tract) were compared between the groups.

[0029]

Herein, the number of the fixed bacteria in the upper respiratory tract was measured as below. Two days after the inoculation of pneumococcus, the mice were sacrificed, and the upper respiratory tract was removed. For the obtained upper respiratory tract, the number of the pneumococcus was determined by the same method as in the measurement of the number of the viable bacteria in the lung.

[0030]

The comparison of the number of the fixed bacteria in the upper respiratory tract

is shown in Fig. 3. Like the result of the number of the bacteria in the lung, in the fermentation product-administrated group, the number of the fixed bacteria in the upper respiratory tract was significantly low compared to the control group.

[0031]

From the above, it can be concluded that the fermentation product of the *Lactobacillus bulgaricus* R-1 strain prevents pneumococcal infection, in particular the infection in the upper respiratory tract and inhibits increase of severity in pneumonia.

Example 3

[0032]

Lactobacillus delbrueckii ssp. *bulgaricus* OLL1073R-1 and *Streptococcus thermophilus* extracted from Meiji Bulgaria Yoghurt as starters were added to a mixture containing dairy products, sugar and raw material water, and fermented. Liquid sugar, sugar, pectin and raw material water were added to this fermentation product (culture) to produce a drink-type yoghurt. When 10 expert panelists ingested the yoghurt, all of the 10 panelists evaluated its flavor as good.

Industrial Applicability

[0033]

The present invention can be used as a novel pharmaceutical composition or a food composition which prevents pneumococcal infection, or prevents or inhibits during the occurrence of the infection.

CLAIMS

1. A prophylactic agent for pneumococcal infection comprising a fermentation product of *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1 as an active ingredient.
2. The prophylactic agent for pneumococcal infection according to Claim 1, containing at least viable bacteria of *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1.
3. The prophylactic agent for pneumococcal infection according to Claim 1 or 2, wherein the fermentation product is a fermentation product with *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1 and *Streptococcus thermophiles*.
4. A method for preventing pneumococcal infection and/or a method for preventing or inhibiting the infection from becoming severe during the occurrence of the pneumococcal infection by ingesting a fermentation product of *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1 with medical practices or non-medical practices.
5. The method according to Claim 4, containing at least viable bacteria of *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1.
6. The method according to Claim 4 or 5, wherein the fermentation product is a fermentation product with *Lactobacillus delbrueckii* ssp. *bulgaricus* OLL1073R-1 and *Streptococcus thermophiles*.