

LACTOBACILLUS RHAMNOSUS CAPABLE OF RELIEVING ALCOHOLIC CHRONIC LIVER INJURY AND APPLICATION THEREOF

This application claims priority to China Application No. 201210046322.0 filed Feb. 28, 2012.

Technical field

The present invention belongs to the field of microbial technology. More specifically, the invention relates to a kind of *Lactobacillus rhamnosus* capable of relieving alcoholic chronic liver injury and its application thereof.

Background Art

Alcohol abuse and alcohol dependence have become increasingly serious public health problems in the world. In China, there is an upward trend in the liver injury due to use of alcohol, and alcohol becomes the second reason of liver injury after virus hepatitis. Damages on the liver caused by alcohol include alcoholic fatty liver, alcoholic hepatitis, alcoholic liver fibrosis, and alcoholic liver cirrhosis. Alcoholic liver injury can also lead to other risks. For example, the liver is unable to filter blood completely which can lead to hyperlipidemia and cardio-cerebrovascular disease; the ability of catabolism of the liver is reduced and is accompanied by diabetes, cholelith disease, kidney disease; acute adiposis hepatica on gestational period and damage on the body's digestive system can also be caused. Therefore, it is of important significance to explore the pathogenesis of alcoholic liver disease and prevention interventions.

Currently, it is believed that the main causes of alcoholic liver injury are the toxic metabolites and metabolic disorders produced in the course of metabolism of ethanol in the liver cell. In detail, causes of alcoholic liver injury are as follows: 1, the toxic effects of acetaldehyde: acetaldehyde promotes lipid peroxidation via interaction with cysteine, glutathione, and vitamin E; as antigen, acetaldehyde combined with a variety of proteins in the liver, stimulates the body to produce antibodies, causing the corresponding immune response and leads to liver cell damage; acetaldehyde can also be combined with important functional groups of enzymes and change the activity of the enzymes and then affect functions of the enzymes. 2, damages caused by free radicals: ethanol can produce a large number of free radicals and reactive oxygen in the process of metabolism, and the free radicals can directly damage the liver cell, and also the free radicals can cause liver cell damage by increasing the sensitivity of liver cell to the lipid peroxidation. 3, induced effect of endotoxin: ethanol intake can cause intestinal flora disorder, simultaneously destroy the integrity of intestinal mucosa's structure and function, and increase the permeability of intestinal mucosa leading to increase of endotoxin level of the blood, while endotoxin can induce the production of a variety of cytokines and damage the liver cells via inflammation factors. Currently,

the main therapeutic methods aiming at alcoholic liver injury include giving up drinking, nutritional therapy, drug treatment, gene therapy, treatment of diseases associated with alcoholic liver disease. The most commonly used method by far is drug treatment, which has certain curative effect and bears many shortcomings as well. For example, many drugs might drive the blood lipid to metabolize more narrowly in the liver, and thus the lipid will accumulate and the functions of liver will be damaged instead; and these drugs are all metabolized in liver, which could further aggravate the burden of liver. Some drugs work slowly or generate severe adverse reactions, even develop drug resistance and toxic and side effect. Therefore, researchers are actively exploring new strategies of treatment and intervention of alcoholic liver disease. Since the probiotics would not produce drug resistance or toxic and side effect, and they have already been widely used for improving human health, especially they also show some pertinence in prevention and treatment of the alcoholic liver disease (shown as figure 5), they are gradually attracting more attention.

Probiotics refer to one kind of viable microorganisms, which, after being taken for certain amount, can promote the growth of native microbial flora of animal or human and then have beneficial influence on the host. Said probiotics mainly include *Lactobacillus*, *Bifidobacterium* and part of *Streptococcus*, etc.. And they generally possess special physiological activity and healthcare function. Said activity and healthcare function include adjusting the intestinal flora of the host, treating diarrhea caused by antibiotics, reducing cholesterol level of the blood lipid, inhibiting infection caused by harmful bacteria such as *E. coli*, *Helicobacter pylori*, etc. In addition, probiotics can effectively remove free radicals, improve the antioxidant activity of the body, adjust the intestinal flora, reduce content of endotoxin and regulate the body's immune system. Such aforementioned functions of probiotics enlighten people that probiotics can play a role in alleviating alcoholic liver injury. So far, there are few reports on protecting liver using probiotics. Therefore, to explore probiotics for being used as health food of diet to alleviate the alcoholic liver injury has an important research significance. As increasing attention pays on alcoholic liver injury and the wide application of probiotics in our daily life, dietary intervention on alcoholic liver disease by using probiotics and its products will have a very broad market prospect.

The prevention and treatment aiming at alcoholic liver injury are mainly focused on compositions of the traditional Chinese medicine in patent applications which have already been open to the public. For example, the patent application CN 101224232A discloses total flavonoids of Puerarin which is extracted from the traditional Chinese herb *radix puerariae lobata*, could inhibit the increase of the intestinal permeability, reduce alcohol concentration of the blood, reduce the absorption of alcohol and the liver damage caused by alcohol. The patent application CN 101961367A discloses a kind of composition of traditional Chinese medicine which could prevent alcoholic liver damage. Said composition consists of polysaccharide of fungus and aqueous extracts of *Silybum marianum*. Such a composition can disintegrate rapidly and has a good intestinal absorption due to its good solubility, and it could also enhance the host

immunity and has an auxiliary protective effect on alcoholic liver damage. Patent applications CN 102058632A and CN 102160637A also respectively disclose the protection effect on alcoholic liver injury of Chinese herbal medicine and the extracts thereof. As for dairy products, for example, the patent application CN 101623032A discloses a kind of milk which has an auxiliary protective effect on alcoholic liver injury. Water-soluble dietary fiber, lecithin and soybean polypeptides etc. are added into said milk, which can improve the liver function, accelerate the metabolism of alcohol, and relieve the alcoholic liver damage. Patent application CN 101328469A discloses *Streptococcus thermophilus* grx02 having protection functions of alcoholic liver injury and proves *Streptococcus thermophilus* grx02 could generate different degree of protection features on acute alcoholic liver damage. However, no lactobacilli strains which could regulate intestinal flora and relieve chronic alcoholic liver damage is involved completely in these patent applications mentioned above.

Therefore, there needs to be a kind of probiotic and related food which can regulate intestinal microflora and relieve chronic alcoholic liver injury.

Content of the present invention

[The technical problem to be solved]

The purpose of the present invention is to provide a kind of *Lactobacillus rhamnosus* with the capability of antioxidation and relieving alcoholic chronic liver injury.

Another purpose of the present invention is to provide an application of said *Lactobacillus rhamnosus*.

[The technical solution]

The present invention is achieved by the following technical solutions.

The present invention relates to *Lactobacillus rhamnosus* CCFM1107, a bacterial strain which was deposited in China General Microbiological Culture Collection Center (CGMCC) located in No.1 yard, Beichen West Road, Chaoyang district, Beijing, on November 29, 2011, with the accession number of CGMCC No.5496.

The present invention also relates to an application of said *Lactobacillus rhamnosus* CCFM1107 in preparing dairy products using bulk starter culture of *Lactobacillus rhamnosus* CCFM1107.

According to the present invention, said dairy products refer to milk, milk powder, milk capsule product or fermented milk which comprise *Lactobacillus rhamnosus* CCFM1107.

According to a preferred embodiment of the present invention, the bulk starter culture of *Lactobacillus rhamnosus* CCFM1107 is prepared according to the following preparation method: The *Lactobacillus rhamnosus* CCFM1107 strain is inoculated in

skimmed milk with sterilization under the temperature of 110°C for 10 min with a ratio of 12% based on the weight of said skimmed milk, then the inoculated skimmed milk is cultured under the temperature of 37°C for 14-16 h till the skimmed milk begin to curd, and then the activated culture is continued for two generations under the same condition, and then the fermented skimmed milk obtained is as the mother starter culture;

Said mother starter culture is inoculated in new skimmed milk with sterilization under the temperature of 110°C for 10 min with a ratio of 3-5% based on the volume of said new skimmed milk, then the inoculated new skimmed milk is cultured under the temperature of 37°C for 14-16 h till the new skimmed milk begin to curd. And said bulk starter culture with a concentration of viable bacterium of $1-3 \times 10^9$ CFU/mL is obtained.

According to another preferred embodiment of the present invention, said bulk starter culture of *Lactobacillus rhamnosus* CCFM1107 is prepared according to the following preparation method:

The *Lactobacillus rhamnosus* CCFM1107 strain is inoculated in MRS liquid medium with a ratio of 1-5% based on the weight of said MRS liquid medium, then the inoculated medium is cultured under the temperature of 37°C for 12-16 h for activation, and the activated culture is continued for two generations under the same condition, and then the activated culture material is inoculated in new MRS liquid medium with a ratio of 2-4% based on the volume of said new MRS liquid medium, then the new inoculated medium is cultured under the temperature of 37°C for 16-18 h, then the medium is centrifuged with speed of 4000 r/min and temperature of 4°C for 15 min, and then the supernatant is removed, then the cell sediment obtained is suspended using sterilized skimmed milk, and then said bulk starter culture with a concentration of viable cell number of $1-3 \times 10^9$ CFU/mL is obtained.

According to another preferred embodiment of the present invention, the milk which comprise *Lactobacillus rhamnosus* CCFM1107 is prepared according to the following procedure:

Raw milk is sterilized via pasteurization with the temperature of 95°C for 20 min or via high temperature sterilization with the temperature of 140°C for 2 s and then is cooled to 4°C, and then said bulk starter culture of *Lactobacillus rhamnosus* CCFM1107 is added to make the concentration of *Lactobacillus rhamnosus* CCFM1107 reaches more than 10^6 CFU/mL and then is kept in 4°C for cold storage, and then the milk which comprise *Lactobacillus rhamnosus* CCFM1107 is obtained.

According to another preferred embodiment of the present invention, the milk powder or milk capsule products which comprise *Lactobacillus rhamnosus* CCFM1107 is prepared according to the following procedure:

Raw milk is sterilized via pasteurization with the temperature of 95 °C for 20 min or

via high temperature sterilization with the temperature of 140 °C for 2 s, the sterilized milk is cooled to 37 °C, and then said bulk starter culture of *Lactobacillus rhamnosus* CCFM1107 is inoculated in the sterilized milk with a ratio of 4% based on the volume of said sterilized milk, then the inoculated sterilized milk is fermented under the temperature of 37 °C for 16 h and then the fermented milk which comprise *Lactobacillus rhamnosus* CCFM1107 is obtained; then, fermented milk which comprise *Lactobacillus rhamnosus* CCFM1107 is inoculated in new sterilized milk with a ratio of 1:3 based on the volume of said new sterilized milk, and then the milk is processed via homogenization, vacuum concentration and spray drying to obtain milk powder which comprise *Lactobacillus rhamnosus* CCFM1107;

The said milk powder which comprise *Lactobacillus rhamnosus* CCFM1107 is enclosed into capsule, and then milk capsule products is obtained.

According to another preferred embodiment of the present invention, the fermented milk which comprise *Lactobacillus rhamnosus* CCFM1107 is prepared according to the following procedure:

Raw milk is sterilized via pasteurization with the temperature of 95°C for 20 min or via high temperature sterilization with the temperature of 140°C for 2 s and then the sterilized milk is cooled to 37°C, and then a ratio of 3-5% of said bulk starter culture of *Lactobacillus rhamnosus* CCFM1107 as well as a ratio of 3-5% of commercial starter cultures used for preparing fermented milk both based on the volume of said sterilized milk are inoculated in the sterilized milk, and then after being mixed uniformly, the inoculated milk is fermented under the temperature of 37°C till the titration acidity reaches to 0.6-0.7% calculated by lactic acid, and then the fermented milk is cooled to 4°C for cold storage, and then the fermented milk which comprise *Lactobacillus rhamnosus* CCFM1107 is obtained.

According to said preferred embodiment of the present invention, wherein said commercial starter cultures are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

According to said preferred embodiment of the present invention, wherein said raw milk is one or more kinds of raw milks selected from skimmed milk, fresh milk and reconstituted milk, said milk refers to cow milk, goat milk or horse milk.

The present invention is to be described in more details as below.

The present invention relates to *Lactobacillus rhamnosus* CCFM1107, a strain which was deposited in China General Microbiological Culture Collection Center (CGMCC) located in No.1 yard, Beichen West Road, Chaoyang district, Beijing, on November 29, 2011, with the accession number of CGMCC No.5496.

The inventor of the present invention screened a strain of probiotics CCFM1107 from strains which were separated and stored by the laboratory, and identified said strain of probiotics CCFM1107 as *Lactobacillus rhamnosus* CCFM1107 via microbiological

characteristics, such as morphological characteristics and culture features, etc., as well as via molecular identification methods for determination the sequence of 16S rDNA. Said strain of probiotics CCFM1107 was deposited in China General Microbiological Culture Collection Center (CGMCC) located in No.1 yard, Beichen West Road, Chaoyang district, Beijing, on November 29, 2011, with the accession number of CGMCC No. 5496.

The morphological characteristics of said *Lactobacillus rhamnosus* CCFM1107 are described below:

Colony characteristics: Said *Lactobacillus rhamnosus* CCFM1107 forms obvious colonies with diameter between 0.5-1.0 mm on MRS culture medium. Said colonies are rounded, with neat edge, milky, transparent, with moist and smooth surface, and no pigment is generated (see figure 1).

Strain morphology: Gram positive, the bacteria is rod-shaped, and the strain exist in forms of single cells, pairs or chains, and the bacteria do not generate spores with rounded terminals (see figure 2).

The culture characteristics of said *Lactobacillus rhamnosus* CCFM1107 are described below:

Said *Lactobacillus rhamnosus* CCFM1107 has a relatively short lag phase and grows into the logarithmic phase at the time of 4 h and the stationary phase at the time of 14~16 h, and gradually declines after 24 h, and then the number of cells begins to reduce.

The liquid culture characteristics of said *Lactobacillus rhamnosus* CCFM1107 are described below:

Said *Lactobacillus rhamnosus* CCFM1107 can grow well in MRS liquid medium, and the medium begins to turn turbid at the time point of 4 h and then the bacteria begin to precipitate at the time point of 8 h and no air bubbles generate when shaking gently. Large amount of bacteria sediment appears after the time point of 12 h. Then milky bacteria sediment increases significantly after 20 h's culture, and said bacteria sediment gather firmly at the bottom of the medium, and the upper medium is very clear, and then the pH value is reduced from 6.2 to 3.8.

In the present invention, said MRS liquid medium can be the medium that is well known to persons skilled in the art with a commodity name as Bacto ® Lactobacilli MRS Broth sold by BD Difco company, said MRS liquid medium can also be other equivalent commercialized medium produced by relevant domestic company.

Said *Lactobacillus rhamnosus* CCFM1107 in the present invention is derived from traditional fermented food and can be used in fermented food since it belongs to the Generally Recognized As Safe (GRAS) strains of the edible microorganism list published by the Ministry of Health.

The present invention also relates to an application of said *Lactobacillus rhamnosus* CCFM1107 in preparing dairy products using bulk starter culture of *Lactobacillus rhamnosus* CCFM1107.

Said bulk starter culture of *Lactobacillus rhamnosus* CCFM1107 is prepared according to the following preparation method:

Generally, first, the pure cultures of *Lactobacillus rhamnosus* CCFM1107 needs to be inoculated repeatedly to recover the activity of strain. Take a small amount of pure cultures and inoculate them into skimmed milk with sterilization under the temperature of 110°C for 10 min. Then the inoculated skimmed milk is cultured under the temperature of 37°C. In the initial hours, mix the skimmed milk and the inoculated pure cultures uniformly by slow vibration, and then the system is cultured statically till the skimmed milk begin to curd. After curding, 1-2mL curding cultures are drew from the bottom by a sterilized straw and added into new sterilized skimmed milk for culturing till the new skimmed milk begin to curd. Repeat several times according to this method to activate the strain sufficiently so as to prepare the mother starter culture.

The *Lactobacillus rhamnosus* CCFM1107 strain which is activated according to the preceding method is inoculated in skimmed milk with sterilization under the temperature of 110°C for 10 min with a ratio of 12% based on the weight of said skimmed milk, then the inoculated skimmed milk is cultured under the temperature of 37°C for 14-16 h till the skimmed milk begin to curd. The activated culture is continued for two generations under the same condition, and the fermented skimmed milk obtained is as the mother starter culture.

Said pasteurization is carried out by using equipment, such as the 145C type of sterilizer sold by the APV company of Britain.

Said skimmed milk is a product that is well known to persons in the art. After check and filtration, the raw milk is preheated to about 38°C, then the preheated raw milk can be divided into two parts consisting of one part of cream and the other part of skimmed milk using a centrifugal separator, such as the closed-type of centrifugal separator manufactured by Alfa Laval company of Sweden.

Said mother starter culture is inoculated in skimmed milk with sterilization under the temperature of 110°C for 10 min with a ratio of 3-5% based on the volume of said skimmed milk. The inoculated skimmed milk is cultured under the temperature of 37°C for 14-16 h till the skimmed milk begin to curd. Then said bulk starter culture with a concentration of viable microorganisms of $1-3 \times 10^9$ CFU/mL is obtained.

The quality of the bulk starter culture directly affects the quality of the fermented dairy products. Therefore, it is necessary to take sensory test on the bulk starter culture and determine whether the bulk starter culture curdles uniformly, whether the tissue of the bulk starter culture is exquisite and compact, whether the bulk starter

culture is flexible, sour and fragrant, and at the same time, without peculiar smell and bubble. It is also necessary to take chemical inspection on the bulk starter culture to determine its acidity, of which, the titratable acidity is generally 90-110 °T. It is also necessary to determine the total number of bacterium of the bulk starter culture according to the conventional methods in the art (See GB 4789.2-2010, National food safety standard, Ministry of Health of the People's Republic of China), and the concentration of viable bacterium of said bulk starter culture of *Lactobacillus rhamnosus* should be $1-3 \times 10^9$ CFU/mL.

Or, said bulk starter culture of *Lactobacillus rhamnosus* CCFM1107 is prepared according to the following preparation method:

The *Lactobacillus rhamnosus* CCFM1107 strain is inoculated in MRS liquid medium with a ratio of 1-5% based on the weight of said MRS liquid medium, and cultured under the temperature of 37 °C for 12-16 h for activation. The activated culture is continued for two generations under the same condition. Then the activated culture material is inoculated in new MRS medium with a ratio of 2-4% based on the volume of said new MRS medium and cultured under the temperature of 37°C for 16-18 h. Then the medium is centrifuged with speed of 4000 r/min and temperature of 4 °C for 15 min. The supernatant is removed and the cell sediment obtained is suspended using sterilized skimmed milk. And said bulk starter culture with a concentration of viable bacterium of $1-3 \times 10^9$ CFU/mL is obtained.

In the present invention, said dairy products refer to milk, milk powder, milk capsule products or fermented milk which comprises *Lactobacillus rhamnosus* CCFM1107.

According to the present invention, the milk which comprises *Lactobacillus rhamnosus* CCFM1107 is prepared according to the following procedure:

Raw milk is sterilized via pasteurization with the temperature of 95°C for 20 min or via high temperature sterilization with the temperature of 140°C for 2 s and then is cooled to 4°C. Said bulk starter culture of *Lactobacillus rhamnosus* is added to make the concentration of bacterium reaches more than 10^6 CFU/mL and then is kept in 4 °C for cold storage. And the milk which comprises *Lactobacillus rhamnosus* CCFM1107 is obtained.

In the present invention, the equipment used for carrying out said pasteurization refers to those equipments that are commonly used in the art as well as are sold extensively on the market. Said pasteurization is carried out by using equipment, such as the 145C type of sterilizer sold by the APV company of Britain.

In the present invention, said high temperature sterilization is carried out by using equipment, such as the PT-20C-R tube-plate combined type of UHT (Ultra High Temperature) sterilizer sold by the Powerpoint International co., LTD of Japan.

Supplementary materials that are commonly used in the art such as sugar, stabilizer, pigment and fruit juice can also be added into said milk which comprises

Lactobacillus rhamnosus CCFM1107.

Said raw milk refers to one or more kinds of raw milk selected from skimmed milk, fresh milk and reconstituted milk. Said milk refers to cow milk, goat milk or horse milk. For example, the skimmed milk could be skimmed cow milk, skimmed goat milk or skimmed horse milk, and the fresh milk could be fresh cow milk, fresh goat milk or fresh horse milk. Said reconstituted milk should be explained as a kind of raw milk which is obtained by blending concentrated whole milk and or whole milk powder and water.

According to the present invention, the milk powder or milk capsule products which comprise *Lactobacillus rhamnosus* CCFM1107 is prepared according to the following procedure:

Raw milk is sterilized via pasteurization with the temperature of 95 °C for 20 min or via high temperature sterilization with the temperature of 140°C for 2 s and then the sterilized milk is cooled to 37 °C. Said bulk starter culture of *Lactobacillus rhamnosus* CCFM1107 is inoculated in the sterilized milk with a ratio of 4% based on the volume of said sterilized milk, then the inoculated sterilized milk is fermented at the temperature of 37 °C for 16 h and then fermented milk which comprises *Lactobacillus rhamnosus* CCFM1107 is obtained; then, said fermented milk which comprises *Lactobacillus rhamnosus* CCFM1107 is inoculated in new sterilized milk with a ratio of 1:3 based on the volume of said new sterilized milk, and then the milk is processed via homogenization, vacuum concentration and spray drying to obtain milk powder which comprises *Lactobacillus rhamnosus* CCFM1107.

Said homogenization is a technology that is frequently used in food production. Homogenization in food production refers to refining the materials via extrusion, strong shock and expansion raised by decompression and making the materials be mixed more uniformly. For example, in dairy industry, homogenizer is used to crush the fat globules in milk into smaller ones so as to make whole product system be more stable. Homogenization is usually carried out by using a homogenizer. Homogenizer is one kind of important processing equipment used in food as well as dairy industry. The homogenizer used in the present invention is the product sold on the market at present, such as the type of GYB40-10S high pressure homogenizer sold by Shanghai Donghua high pressure homogenizer Factory.

According to the present invention, said vacuum concentration is a technology that is frequently used in food production. There is no difficulty for persons skilled in the art to choose the temperature of concentration and the vacuum degree according to the properties of the materials. The equipment used for vacuum concentration in the present invention is the product sold on the market at present, such as the vacuum concentration cauldron sold by Yangzhou City Food Machinery Factory.

According to the present invention, said spray drying is a technology that is frequently used in food production. There is no difficulty for persons skilled in the art to choose

the temperature and duration of drying according to the properties of the materials. The equipment used for spray drying in the present invention is the product sold on the market at present, such as the experimental spray dryer sold by Shanghai Wodi Technology Co. Ltd.

According to the present invention, said milk powder which comprises *Lactobacillus rhamnosus* CCFM1107 is enclosed into capsules and then milk capsule products is obtained.

According to the present invention, said capsules refer to those capsules sold on the market of medicine and food at present.

According to the present invention, the fermented milk which comprises *Lactobacillus rhamnosus* CCFM1107 is prepared according to the following procedure:

Raw milk is sterilized via pasteurization at the temperature of 95°C for 20 min or via high temperature sterilization at the temperature of 140°C for 2 s and then the sterilized milk is cooled to 37°C, and then a ratio of 3-5% of said bulk starter culture of *Lactobacillus rhamnosus* CCFM1107 as well as a ratio of 3-5% of commercial starter cultures used for preparing fermented milk both based on the volume of said sterilized milk are inoculated in the sterilized milk, and then after being mixed uniformly, the inoculated milk is fermented at the temperature of 37 °C till the titration acidity reaches to 0.6-0.7% calculated by lactic acid, and then the fermented milk is cooled to 4 °C for cold storage, and then the fermented milk which comprise *Lactobacillus rhamnosus* CCFM1107 is obtained.

Said commercial starter cultures in the present invention refers to *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, such as the products of Danisco company of the United States or Chr. Hansen company of Denmark.

Lactobacillus bulgaricus (*Lactobacillus delbrueckii* subsp. *bulgaricus*) widely used in the process of fermented milk production at present belongs to the genus of *Lactobacillus*. It was named as *Lactobacillus delbrueckii* subsp. *bulgaricus* (*Lactobacillus bulgaricus* for short) by microbiologist due to its characteristics including the producing area of the strain, microbial properties and high efficiency.

Streptococcus thermophilus is one kind of important starter culture for preparing fermented milk. It is widely used for producing fermented dairy products including yogurt and cheese. *Streptococcus thermophilus* also bears some functional activities, such as producing extracellular polysaccharide, bacteriocin and vitamins.

Animal experiments showed that *Lactobacillus rhamnosus* CCFM1107 in the present invention can improve the liver function and anti-oxidation index of the mice with alcoholic liver injury as well as alleviate endotoxaemia and regulate intestinal flora distribution. Said *Lactobacillus rhamnosus* CCFM1107 can effectively alleviate the alcoholic liver disease with equivalent even better effects to the commonly used

commercialized drug on the market with a Chinese name “KUIHUA HUGAN PIAN” which is produced by Sunflower pharmaceutical Co.,Ltd. of Heilongjiang Province.

[Advantageous Effects]

Lactobacillus rhamnosus CCFM1107 in the present invention has high antioxidant activity. The clearance rates of diphenyl picrylhydrazyl (DPPH) free radical are 93.51% and 89.66% respectively for intact cells and cell free extracts of *Lactobacillus rhamnosus* CCFM1107 at a cell concentration of 10^{10} CFU/mL. The clearance rates of hydroxyl free radical are 94.16% and 93.87% respectively for intact cells and cell free extracts of *Lactobacillus rhamnosus* CCFM1107 at a cell concentration of 10^{10} CFU/mL. Intact cells and cell free extracts of said *Lactobacillus rhamnosus* CCFM1107 both have certain reducing capacity. The reducing capacities of intact cells and cell free extracts at a cell concentration of 10^{10} CFU/mL are respectively equivalent to that of 392.07 μ mol/L and 373.91 μ mol/L cysteine hydrochloride. *Lactobacillus rhamnosus* CCFM1107 in the present invention also has the capacity of inhibiting lipid peroxidation. The inhibition rates of lipid peroxidation are 84.52% and 81.18% respectively for intact cells and cell free extracts at a cell concentration of 10^{10} CFU/mL. Said *Lactobacillus rhamnosus* CCFM1107 can tolerate a concentration of 0.35% of bile salts, a concentration of 8% of NaCl, and pH value of 3.

The results of the animal experiments showed that *Lactobacillus rhamnosus* CCFM1107 in the present invention can improve the liver function and anti-oxidation index of the mice with alcoholic liver injury as well as alleviate endotoxaemia and regulate intestinal flora distribution. Said *Lactobacillus rhamnosus* CCFM1107 can effectively alleviate the alcoholic liver disease with equivalent even better effects to the commonly used commercialized drug on the market with a Chinese name “KUIHUA HUGAN PIAN” which is produced by Sunflower pharmaceutical Co.,Ltd. of Heilongjiang Province.

The strain of *Lactobacillus rhamnosus* CCFM1107 was deposited in China General Microbiological Culture Collection Center (CGMCC) located in No.1 yard, Beichen West Road, Chaoyang district, Beijing on November 29, 2011 with an access number of CGMCC 5496.

Description of Figures:

Fig.1 shows the colony morphology of *Lactobacillus rhamnosus* CCFM1107;

Fig.2 shows the morphology with gram positive stain of *Lactobacillus rhamnosus* CCFM1107 (1000 $\times$);

Fig.3 shows the growth curves of *Lactobacillus rhamnosus* CCFM1107 in MRS liquid medium at the temperature of 37 $^{\circ}$ C under the anaerobic culture condition;

Fig.4 shows the HE staining observation results of groups of mice liver pathological sections (200 $\times$), A stands for the group of blank, B stands for the group of model, C

stands for the group of drug, D stands for the group of intervention which is being taken gavage of *Lactobacillus rhamnosus* CCFM1107, E stands for the group of negative control which is being taken gavage of *Lactobacillus plantarum* N-9;

Fig.5 shows the relationship between the causes of alcoholic liver damage and the health benefits of probiotics.

Detailed description of the preferred embodiments

The embodiments below are used for better understanding of the invention. Unless noted otherwise, the equipments and measuring methods etc. mentioned in these embodiments are as described in the preceding part of the description, and specifically not repeated.

Embodiment 1: Sequence identification on the 16S rDNA of the *Lactobacillus rhamnosus* CCFM1107 in the present invention

Lactobacillus rhamnosus CCFM1107 was inoculated in MRS liquid medium and cultured for 18 h with temperature of 37°C. Then 1 mL of bacterial liquid was gathered to extract the genome DNA using bacterial genome DNA extraction kit according to the instruction of the kit. Then a PCR amplification reaction with a volume of 50 μ L was carried out using the genome DNA as a template and using the universal primers for bacteria identification disclosed in the paper (Critical Evaluation of Two Primers Commonly, informs the for Amplification of Bacterial 16S rRNA Genes. Applied and Environmental Microbiology, 2008, 74 (8) 2461-2470) as primers, and then the amplification product was purified, recycled and sequenced.

The sequencing of the PCR amplification product of the target gene was done by Sangon Biotech (Shanghai) Co., Ltd. Compared with the nucleic acid database of NCBI, the sequencing results of 16S rDNA of *Lactobacillus rhamnosus* CCFM1107 indicated that *Lactobacillus rhamnosus* CCFM1107 in the present invention bore a homology as high as 99% to many kinds of *Lactobacillus rhamnosus*, such as *Lactobacillus rhamnosus* strain HT2, *Lactobacillus rhamnosus* strain 20300 and *Lactobacillus rhamnosus* strain NM94-5 etc.. Therefore, the CCFM1107 strain in the present invention was identified as *Lactobacillus rhamnose* and was named as *Lactobacillus rhamnosus* CCFM1107. It was deposited in China General Microbiological Culture Collection Center (CGMCC) located in No.1 yard, Beichen West Road, Chaoyang district, Beijing on November 29, 2011 with an access number of CGMCC 5496.

Embodiment 2: Determination of microbiological properties of *Lactobacillus rhamnosus* CCFM1107

Lactobacillus rhamnosus CCFM1107 was inoculated in MRS liquid medium with a volume ratio of 5%, then the pH of the medium is measured by pH meter and the OD₆₀₀ value was measured using spectrophotometer at 600 nm at the time points of 0

h, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, 12 h, 14 h, 16 h, 18 h, 20 h, 22 h and 24 h, respectively. The pH meter used in the present invention was the product sold by Mettler-Toledo (Shanghai) Co.,Ltd., and the ultraviolet spectrophotometer was the product sold by UNICO(Shanghai)Instruments Co.,Ltd..

Based on the value of OD₆₀₀ and pH, a growth curve of *Lactobacillus rhamnosus* CCFM1107 in the MRS liquid medium could be got, which is shown as figure 3. In the MRS liquid medium, *Lactobacillus rhamnosus* CCFM1107 had a relatively short lag phase and grew into the logarithmic phase at the time of 4 h and the stationary phase at the time of 14~16 h. The pH of the medium declined as the culture time extended and kept essentially constant when grew into the stationary phase. The pH declined from the original value 6.13 to 3.86 after 24 h's culture. The concentration of viable *Lactobacillus rhamnosus* CCFM1107 in the medium was 6.8×10^8 CFU/mL at the time of 24 h.

Embodiment 3: Antioxidant capacity of *Lactobacillus rhamnosus* CCFM1107

Firstly, intact cells and cell free extracts of *Lactobacillus rhamnosus* CCFM1107 were prepared.

After activated culture, *Lactobacillus rhamnosus* CCFM1107 of the present invention was inoculated in MRS liquid medium and cultured for 24 h under the condition of 37°C. Then supernatant and bacteria sediment were got via centrifugation with speed of 6000 r/min and temperature of 4 °C. The bacteria sediment was re-suspended in sterile normal saline after being washed twice by sterile normal saline. The cell concentration was adjusted to about 10^9 CFU/mL. Then the bacterial cell suspension obtained was divided into two groups. One was as intact cells (IC) and the other was used to prepare cell free extracts (CFE). Said cell free extracts was prepared as follows: firstly, the bacterial cell suspension was processed for 30 min under the condition of 200W and 4°C via ultrasonication using a ultrasonic processor (Sonics&Materials company, VCX500 type) with 5 s for each treatment and 5 s for each interval. The processed bacterial cell suspension was examined under the microscope to make sure no intact bacterial cells was residual. The processed bacterial cell suspension was centrifuged with speed of 6000 r/min and temperature of 4°C, and the supernatant obtained at last was the CFE.

Afterwards, the determination of antioxidant capacities including the clearance rate of DPPH free radical and hydroxyl free radical, reduction activity and inhibition rate of lipid peroxidation of *Lactobacillus rhamnosus* CCFM1107 was carried out, and results obtained were listed in Table1.

(1) the ability of removing DPPH free radicals

DPPH (1, 1 Diphenyl - 2 - picrylhydrazyl radical) free radical is a common effective substance used to screen and evaluate antioxidants. It's a stable organic free radical bearing a single electron and is purple in the alcohol solution. It has strong absorption

at 517 nm. The absorption will weaken since materials that could clear away the DPPH free radical is added in. Then the antioxidant capacity of the material being tested could be determined through the change of absorption from strong to weak. The present embodiment determined the ability of removing DPPH free radicals of both intact cells and cell free extracts of *Lactobacillus rhamnosus* CCFM1107 according to the improved MEEI - YN LIN and FEN - JUAN CHANG method (Antioxidative effect of intestinal bacteria *Bifidobacterium longum* ATCC 15708 and *Lactobacillus acidophilus* ATCC 4356. Digestive Diseases and Sciences, 2000, 45 (8): 1617-1622). The clearance rate of DPPH free radical was figured out based on the method of calculation disclosed in this paper.

(2) the ability of removing hydroxyl free radical

Hydroxyl free radical, which is a major reason causing oxidative damage in the body, bears the highest activity and the strongest antioxidative ability, and also, has relatively strong combining ability to DNA, proteins and lipids. In the present embodiment, the hydroxyl free radicals HO $\cdot$ was generated via Fenton reaction and phenanthroline - Fe²⁺ was as the redox indicator of this reaction. When scavenger of HO $\cdot$ was added in, the content of HO $\cdot$ was reduced and at the same time the content of Fe²⁺ increased and then the solution turned red. Detailed method was according to what was disclosed in the paper (John M. C. GUTTERIDGE, Ferrous-salt-promoted damage to deoxyribose and benzoate. The increased effectiveness of hydroxyl-radical scavengers in the presence of EDTA, *Biochemical Journal*. 1987, 243:709-714). The ability of removing hydroxyl free radical was expressed using the term “clearance of the hydroxyl free radical”, which was figured out based on the method of calculation disclosed in this paper.

(3) the determination of reduction activity

Reduction activity mainly means that some enzymes (such as catalase, NADH oxidase, NADH peroxidase) and the some nonenzymic complexes (such as vitamin C, vitamin E, glutathione) have the ability of reducing oxygen free radicals and chelating Fe²⁺ so as to reduce oxidation reaction. The present embodiment determined the reduction activity of both intact cells and cell free extracts of *Lactobacillus rhamnosus* CCFM1107 according to the improved Meei - Yn Lin and Chyuan - Liang Yen method (Antioxidative ability of lactic acid bacteria. *Journal of Agricultural and Food Chemistry*, 1999, shall divide 60-1466). The reduction activity was expressed using the term “reducing power”, which was figured out based on the method of calculation disclosed in this paper, and it was equivalent to the concentration of cysteine hydrochloride.

(4) the capacity of inhibiting lipid peroxidation

Lipid peroxidation mainly refers to a series of free radical reactions that occur among the unsaturated fatty acids of biological membrane induced by oxygen free radicals. The final products of lipid peroxidation include malondialdehyde (MDA), which

could destroy the biological macromolecules such as protein, nucleic acid and cause aging and various diseases of the body. Therefore, determine the content of MDA, to some certain extent, can reflect the degree of damage of lipid peroxidation, and it is also an acknowledged index reflecting lipid peroxidation. The present embodiment determined the capacity of inhibiting lipid peroxidation of both intact cells and cell free extracts of *Lactobacillus rhamnosus* CCFM1107 according to the improved M.Y. LIN and C. L. YEN method (Reactive oxygen species and lipid peroxidation product-scavenging ability of yogurt organisms. *Journal of Dairy Science*, 1999, 82:1629-1634). The capacity of inhibiting lipid peroxidation was expressed using the term “rate of inhibiting lipid peroxidation”, which was figured out based on the method of calculation disclosed in this paper.

Results of the clearance rate of DPPH free radical and hydroxyl free radical, reducing power and inhibition rate of lipid peroxidation of *Lactobacillus rhamnosus* CCFM1107 stated above were collected in table 1.

Table 1. Antioxidant capacity of *Lactobacillus rhamnosus* CCFM1107

Index of antioxidant capacity	clearance rate of DPPH free radical/%	clearance rate of hydroxyl free radical/%	reducing power / equivalent to the concentration of cysteine hydrochloride ($\mu\text{mol/L}$)	inhibition rate of lipid peroxidation /%
intact cells	93.51 $\pm$ 3.57	94.16 $\pm$ 5.64	392.07 $\pm$ 7.15	84.52 $\pm$ 3.69
cell free extracts	89.66 $\pm$ 4.02	93.87 $\pm$ 2.38	373.91 $\pm$ 6.36	81.18 $\pm$ 4.85

It could be drawn from the above table that *Lactobacillus rhamnosus* CCFM1107 showed relatively high activity in clearing free radicals, inhibiting lipid peroxidation and reducing power. To sum up, the *Lactobacillus rhamnosus* CCFM1107 strain has relatively high antioxidant activity among the strains for screening.

Embodiment 4: The tolerance experiment of bile for *Lactobacillus rhamnosus* CCFM1107

Add ox-bile salts in MRS liquid medium to make the mass concentration of ox-bile salts to 0.0%, 0.10%, 0.20%, 0.30%, 0.35%, 0.40% and 0.45%. Then inoculating the *Lactobacillus rhamnosus* strain CCFM1107, which is 5% of the volume of MRS on sterilized liquid MRS. Culturing it under 37°C for 24 hours and observing the growth condition of strain on various MRS, then measure OD₆₀₀ value under 600nm. The growth condition of *Lactobacillus rhamnosus* CCFM1107 on MRS medium of various concentration of bile can be seen in Table 2. It is known that the inhibition of bile to this strain depends on the concentration of bile salts as well as the characters of strains. The amount of bile salts in human intestinal is 0.03%-0.05%. Only the strains which can grow and metabolize under normal concentration of physiological bile can

survive in intestinal. From the results of Table 2, it can be seen that the *Lactobacillus rhamnosus* CCFM1107 in this invent can grow in the medium with the concentration of bile salts of 0.35%. So it can be concluded that the *Lactobacillus rhamnosus* CCFM1107 has high tolerance to bile salts.

Table 2. The growth condition of *Lactobacillus rhamnosus* CCFM1107 on MRS medium of various concentration of bile

The concentration of bile (%)	Growth condition
0.00	++
0.10	++
0.20	+
0.30	+
0.35	+
0.40	-
0.45	-

Note: ++ indicates good growth, that is to say culture is very turbid and the precipitation of bacteria can be seen.

+ indicates weak growth, that is to say culture is little turbid and naked eyes can see little precipitation of bacteria.

- indicates no growth, that is to say no precipitation of culture and the bacterial culture is clear.

Embodiment 5: The tolerance experiment of NaCl for *Lactobacillus rhamnosus* CCFM1107

Add NaCl in MRS liquid medium to make the mass concentration of NaCl to 0%, 2%, 4%, 6%, 7%, 8% and 9%. Then inoculating the *Lactobacillus rhamnosus* strain CCFM1107, which is 5% of the volume of MRS on sterilized liquid MRS. Culturing it under 37°C for 24 h and observing the growth condition of strain on various MRS, then measure OD₆₀₀ value under 600nm. The results are shown in Table 3.

It can be seen from the Table 3 that *Lactobacillus rhamnosus* CCFM1107 grows well under NaCl concentration of 7%, and grows weakly under 8%. It cannot grow under NaCl concentration of 9% and above, indicating the tolerant concentration of NaCl for CCFM1107 is 8%.

Table 3. The growth condition of *Lactobacillus rhamnosus* CCFM1107 on MRS medium of various concentration of NaCl

NaCl (%)	Growth condition
0	+++
2	+++
4	+++
6	++
7	++

8	+
9	-

Note: ++ indicates good growth, that is to say culture is very turbid and the precipitation of strain can be seen.

+ indicates weak growth, that is to say culture is little turbid and naked eyes can see little precipitation of bacteria.

- indicates no growth, that is to say no precipitation of bacteria and the bacterial culture is clear.

Embodiment 6: The tolerance experiment of various pH for *Lactobacillus rhamnosus* CCFM1107.

Add 1M HCl solution in MRS liquid medium to make the pH values be 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 6.2. Then inoculating the *Lactobacillus rhamnosus* strain CCFM1107, which is 5% of the volume of MRS on sterilized liquid MRS. Culturing it under 37°C for 24 h and observing the growth condition of strain in various MRS, then measure OD₆₀₀ value under 600nm. The results are shown in Table 4.

The normal pH of human gastric juice is 1.5-4.5, and it fluctuates with personal diet construction. Normally the pH of gastric juice is 3. If the strain can survive in intestinal, it should be tolerant to acid. It is analyzed from the results of Table 4, *Lactobacillus rhamnosus* CCFM1107 can grow in condition of pH 3, meaning that it has strong capability of survival and growth under low pH.

Table 4. The growth condition of *Lactobacillus rhamnosus* CCFM1107 on MRS medium of various pH

The Value of pH	Growth condition
1.5	-
2.0	-
2.5	-
3.0	+
3.5	++
4.0	++
6.2	++

Note: ++ indicates good growth, that is to say culture is very turbid and the precipitation of strain can be seen.

+ indicates weak growth, that is to say culture is little turbid and naked eyes can see little precipitation of bacteria.

- indicates no growth, that is to say no precipitation of bacteria and the bacterial culture is clear.

Embodiment 7: The animal experiment of relieving chronic alcoholic liver damage by *Lactobacillus rhamnosus* CCFM1107

Build the model of mice chronic alcoholic liver damage according to the methods of

F.Sun and M. L. Xie (Inhibitory effect of osthole on alcohol-induced fatty liver in mice. *Digestive and Liver Disease*, 2009, 41:127-133). And feed mice probiotics to analysis the relieving function of *Lactobacillus rhamnosus* CCFM1107 to chronic alcoholic liver injury.

50 Kunming mice of clean grade, male, weighing 18±2g, are purchased from Shanghai SLRC Laboratory animal Ltd; Permission license number: SCXK (Hu) 2007-0005.

Feed the mice with rod feed in standard formula, and mice can drink freely. Mice are fed in clean grade animal experiment labs in College of Pharmacy, Jiangnan University. Temperature: 20-23°C; Humidity: 50%-60%.

After 3 days of adaptation feed, the mice are divided into 5 groups randomly, and the group conditions can be seen in Table 5. Every group has 10 mice and they are feed as Table 5. They are fed twice every day with alcohol in the morning and drug or probiotics of this invent in the afternoon.

Table 5. The animal experiment of relieving chronic alcoholic liver damage by *Lactobacillus rhamnosus* CCFM1107

Group	Feed Methods
Blank	Skim Milk(am)+ Skim Milk(pm)
Model	Alcohol(am)+Skim Milk(pm)
Drug	Alcohol(am)+ Liver tablets(pm)
Intervention	Alcohol(am)+CCFM1107(pm)
Contrast	Alcohol(am)+N-9(pm)

Note: am means morning, pm means afternoon, CCFM1107 is *Lactobacillus rhamnosus* CCFM1107 in this invent with high antioxidant while N-9 is with low antioxidant as a negative control.

The concentration of fed alcohol is added gradually according to the order: 20%-25%-30%-35%-40%. It will added to 40% (v/v) in two weeks and maintain this concentration until the experiment is over.

The positive control drug is drug on the market with a Chinese name “KUIHUA HUGAN PIAN” which is produced by Sunflower pharmaceutical Co.,Ltd. of Heilongjiang Province and is transferred to mice corresponding concentration; Make *Lactobacillus rhamnosus* CCFM1107 in this invent into lyophilized powder, and recover it in 37°C water for 30 minutes before feed every time. The concentration is about 10⁹ CFU/mL. All samples are gavaged as 10mL/KgBW for 3 months. After the last gavage, food is forbidden for 24 hours and eyeballs are removed quickly to collect blood. Dissect the mice to collect samples of liver and intestinal fecal and measure the indexes like AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), TG (Triglycerides), TC (Total cholesterol).

The measurement kits of AST, ALT, TG, TC are provided by Changchun Huili Biotechnical Ltd. And the measurement kits of MDA (Malondialdehyde), GSH (Glutathione), SOD (Superoxide dismutase) and GSH-PX (Glutathione peroxidase) are provided by Nanjing Jiancheng Bioengineer research agency. The test results are

shown in table 6 to table 13. All data are analyzed by software SPSS 16.0 to make statistical analysis. All data are shown in form of $\bar{x} \pm s$, using One-way ANOVA to do significant test.

Liver index is the ratio of liver weigh and body weight and it can reflect the health of liver on some degree. As the lesions occur, the liver will shrink or swell, the liver index will change as well. The liver index of five groups of mice are shown in Table 6: the liver index of model group is bigger than that of blank remarkably; after the treatment, the liver index of group drug and intervention of *Lactobacillus rhamnosus* CCFM1107 declines and drug group and model group are of significant difference.

Table 6. The effect of *Lactobacillus rhamnosus* CCFM1107 to mice liver index ($\bar{x} \pm s$, n=10)

Group	Liver Index (%)
Blank	2.98±0.47
Model	3.74±0.52 ^a
Drug	3.11±0.39 ^b
Intervention	3.31±0.47
Contrast	3.59±0.36 ^a

Note: ^a indicates a significant difference ($P < 0.05$) compared with blank group, ^b indicates a significant difference ($P < 0.05$) compared with model group.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are mainly in cytoplasm of liver cells. When the liver is injured, the transaminase in cells will enter in blood, elevate the level of ALT and AST in blood. AST is also in mitochondria, when liver is seriously injured, AST will be released from mitochondria to blood and enhance the level of AST in serum. So the activity of ALT and AST in serum is the most acute marker of liver injure induced by alcohol.

The Table 7 indicates that the alcohol inducement can elevate ALT and AST amount in serum remarkably, while the drug group and intervention group of *Lactobacillus rhamnosus* CCFM1107 decline them to the level in that of blank group.

Table 7. The effect of *Lactobacillus rhamnosus* CCFM1107 to mice transaminases in serum ($\bar{x} \pm s$, n=10)

Group	AST(U/L)	ALT(U/L)
Blank	41.65±10.02	27.49±6.45
Model	73.99±7.89 ^a	36.03±7.36 ^a
Drug	43.92±9.32 ^b	25.25±3.01 ^b
Intervention	47.88±8.24 ^b	26.49±5.29 ^b
Contrast	74.80±11.14 ^a	37.51±8.84 ^a

Note: ^a indicates a significant difference ($P < 0.05$) compared with blank group, ^b indicates a significant difference ($P < 0.05$) compared with model group.

The liver injured by alcohol can make steatosis and produce lipid vesicles. So the content of blood lipids are measured and the results are listed in Table 8. Compared

with blank group, the level of blood lipids are elevated remarkably while the drug group and intervention group of *Lactobacillus rhamnosus* CCFM1107 decline them to the normal level.

Table 8. The effect of *Lactobacillus rhamnosus* CCFM1107 to mice blood lipids ($\bar{x} \pm s$, n=10)

Group	TG (mmol/L)	TC (mmol /L)
Blank	2.24±0.49	2.33±0.51
Model	3.74±0.65 ^a	3.83±0.61 ^a
Drug	2.17±0.45 ^b	2.49±0.65 ^b
Intervention	2.32±0.63 ^b	2.80±0.59 ^b
Contrast	3.37±0.72 ^a	3.80±0.72 ^a

Note: ^a indicates a significant difference ($P < 0.05$) compared with blank group, ^b indicates a significant difference ($P < 0.05$) compared with model group.

Free radicals and its induced lipid peroxidation is also a cause to liver injury. Malondialdehyde (MDA) is the product of lipid peroxidation, and its amount can reflect the degree of lipid peroxidation in vivo and the degree of liver injured indirectly. Glutathione peroxidase (GSH-PX), together with superoxide dismutase (SOD) can clear active oxygen using glutathione (GSH) as substrate to alleviate and prohibit the oxidation of active oxygen. It can be analyzed from Table 9 and Table 10, the amount of GHS, GHE-PX and SOD are declined greatly induced by alcohol while the concentration of MDA elevates. The amount of GHS, GHS-PX and SOD are enhanced and MDA declined in drug group and intervention group of *Lactobacillus rhamnosus* CCFM1107. Intervention group of *Lactobacillus rhamnosus* CCFM1107 has better effect than that of drug group, it makes the concentration of GHS higher than the normal and GSH-PX and SOD are improved remarkably ($P < 0.05$).

Table 9. The effect of *Lactobacillus rhamnosus* CCFM1107 to mice MDA and GHS in liver homogenates ($\bar{x} \pm s$, n=10)

Group	MDA(nmol/mg protein)	GSH(mg/g protein)
Blank	6.01±1.74	9.06±2.41
Model	12.92±2.91 ^a	5.75±1.67 ^a
Drug	7.33±2.05 ^b	6.99±1.92
Intervention	6.48±2.28 ^b	9.85±2.17 ^b
Contrast	11.16±2.77 ^a	6.11±2.41 ^a

Note: ^a indicates a significant difference ($P < 0.05$) compared with blank group, ^b indicates a significant difference ($P < 0.05$) compared with model group.

Table 10. The effect of *Lactobacillus rhamnosus* CCFM1107 to mice SOD and GSH-PX in liver homogenates ($\bar{x} \pm s$, n=10)

Group	SOD(U/mg protein)	GSH-PX(activity unit)
Blank	105.22±20.97	214.37±23.79

Model	71.88±12.43 ^a	176.32±19.24 ^a
Drug	92.66±14.52 ^b	179.01±16.03 ^a
Intervention	97.22±13.84 ^b	203.14±24.36
Contrast	68.58±15.17 ^a	205.55±18.17 ^b

Note: ^a indicates a significant difference ($P < 0.05$) compared with blank group, ^b indicates a significant difference ($P < 0.05$) compared with model group.

It can be indicated from Table 11, the intake of alcohol not only elevate amount of blood lipids but also its concentration in liver. Correspondingly, the amount of TG and TC in liver homogenates of drug group and intervention group of *Lactobacillus rhamnosus* CCFM1107 are declined. Intervention group of *Lactobacillus rhamnosus* CCFM1107 has better effect on declining TG while the drug group on TC.

Table 11. The effect of *Lactobacillus rhamnosus* CCFM1107 to mice TG and TC in liver homogenates ($\bar{x} \pm s$, n=10)

Group	TG(mmol/L)	TC(mmol/L)
Blank	0.83±0.09	1.34±0.12
Model	1.28±0.23 ^a	2.26±0.27 ^a
Drug	0.99±0.13 ^b	1.53±0.21 ^b
Intervention	0.88±0.13 ^b	1.80±0.26 ^{a,b}
Contrast	1.23±0.17 ^a	2.25±0.33 ^a

Note: ^a indicates a significant difference ($P < 0.05$) compared with blank group, ^b indicates a significant difference ($P < 0.05$) compared with model group.

One of the main physical function of probiotics is adjusting intestinal flora. When liver is injured, the intestinal flora will change and the intake of probiotics can play an important role in maintain and stabilize micro ecological environment in intestinal. Collect excrement from mice intestinal into sterile tubes and weigh it. And gradient diluted by sterile buffer (1L PBS buffer containing 0.5g Cysteine hydrochloride, 0.5mL Twain 80 and 0.5g Agar, adjusting pH to 7.4-7.6) to 10 multiples. Choose the suitable multiple dilution 100 μ L on selective medium such as *Lactobacillus* selective modified MC medium (Qingdao Haibo Biotechnology Ltd), *Bifidobacterium* selective modified TPY medium (Qingdao Haibo Biotechnology Ltd), *Enterobacter* selective modified VRBDA medium (Qingdao Haibo Biotechnology Ltd) and *Enterococcus* selective modified EC medium (Qingdao Haibo Biotechnology Ltd) to count number. *Lactobacillus* and *Bifidobacterium* are anaerobic cultured in 37°C, *Enterobacter* is aerobic cultured in 37°C, *Enterococcus* is aerobic cultured in 42°C. After 48 h, counting the colony forming unit (CFU) of 1g of excrement sample, and the results are shown in form of log₁₀ (CFU/g intestinal excrement). The endotoxin in serum are analyzed by Enzyme-linked immunosorbent assay according to the operation of ELISA kit (Cusabio Company). These results are listed in Table 12 and Table 13.

Table 12. The effect of *Lactobacillus rhamnosus* CCFM1107 to mice micro flora in intestinal

($\bar{x} \pm s$, n=10)

Group	<i>Enterococcus</i>	<i>Enterobacter</i>	<i>Lactobacillus</i>	<i>Bifidobacterium</i>
Blank	6.10±0.17	6.13±0.17	8.53±0.20	9.35±0.15
Model	6.51±0.23	7.59±0.20 ^a	7.90±0.21	8.14±0.26 ^a
Drug	6.30±0.19	7.03±0.24 ^{a,b}	8.06±0.27	8.32±0.17 ^a
Intervention	4.48±0.26 ^{a,b}	4.52±0.20 ^{a,b}	8.99±0.28 ^b	9.89±0.16 ^{a,b}
Contrast	5.54±0.20 ^b	5.32±0.13 ^{a,b}	8.72±0.22 ^b	9.17±0.21 ^b

Note: ^a indicates a significant difference ($P < 0.05$) compared with blank group, ^b indicates a significant difference ($P < 0.05$) compared with model group.

Table 13. The effect of *Lactobacillus rhamnosus* CCFM1107 to serum endotoxin in mice ($\bar{x} \pm s$, n=10)

Group	The Amount of Endotoxin in Serum(pg/mL)
Blank	28.29±6.48
Model	66.14±12.47 ^a
Drug	54.35±13.24 ^a
Intervention	27.93±12.77 ^b
Contrast	36.28±13.12 ^b

Note: ^a indicates a significant difference ($P < 0.05$) compared with blank group, ^b indicates a significant difference ($P < 0.05$) compared with model group.

Analysis from Table 12 and 13, compared with blank group, the number of *Enterobacter* are remarkably increased while *Lactobacillus* and *Bifidobacterium* are greatly declined in alcohol-treated groups; in probiotics groups, no matter intervention group or contrast group, the number of *Lactobacillus* and *Bifidobacterium* is far higher than that of alcohol-treated groups. In model group, they are higher than the normal level while the number of *Enterococcus* and *Enterobacter* remarkably decline. However, the drug group has little effect on intestinal micro flora, they are on the same level of that of model group. Correspondingly, the amount of endotoxin in serum in model group and drug group are higher than in blank group, and the difference is very significant ($P < 0.05$). Intaking of probiotics can decline the inner endotoxin level, and the model group with *Lactobacillus rhamnosus* CCFM1107 has little inner endotoxin in serum than blank group.

Take the liver of same position from every group of mice to evaluate the alleviation of probiotics *in vivo* to liver injure induced by alcohol. The pathological section of mice HE using hematoxylin -eosin staining are shown in Figure 4. The blank group (Fig. 4A): the liver cell cord has complete construction and is radical; the construction of lobule is clear, and the liver cells have obvious boundaries and uniform cytoplasm; there is no fat holes and inflammatory invasion. The model group (Fig. 4B): Fatty degeneration is obvious with large areas of fat holes, and liver cells are swelled and deformed with turbid cytoplasm and net construction. Few nuclear are condensed and inflammatory invasion appear. The drug group (Fig. 4C): it is improved than the model group with little fat holes and mild cell inflammatory invasion and mild

unclear condensation. CCFM1107 intervention group (Fig. 4D): boundaries of lobule are clear, cell plates are normal and ranged in order. Negative control *Enterobacter* N-9 (Fig. 4E): there are many fat holes and swelling liver cells with net construction and mild inflammatory invasion.

In conclusion, in the model of mice chronic alcoholic liver injury, *Lactobacillus rhamnosus* CCFM1107 can decline the activities of serum transaminases and amount of lipids to enhance antioxidant capability of mice, to inhibit the formation of radicals and adjust the micro flora distribution in intestinal as well as decline amount of toxin in serum to prevent mice liver from fatty degradation. From the indexes of serum and liver, *Lactobacillus rhamnosus* CCFM1107 in this invent has good capacity to alleviate the chronic alcoholic liver injury and it can be applied to the development of function food and related medicines.

Applied Example 1: Using *Lactobacillus rhamnosus* CCFM1107 to make milk containing CCFM1107

Frist of all, make bulk starter culture of *Lactobacillus rhamnosus* CCFM1107.

The original *Lactobacillus rhamnosus* CCFM1107 strain is inoculated in skimmed milk sterilized under the temperature of 110 °C for 10 min using 145C antiseptic sold by SPX/APV company with a ratio of 12% based on the weight of said skimmed milk. Then cultured it in 37°C for 4 h till the skimmed milk begin to curd. And then the activated culture is continued for two generations under the same condition. The fermented skimmed milk obtained is as the mother starter culture.

The mother starter culture, 5% volume of skim milk, is inoculated on skim milk, which has been sterilized in 110°C for 10 min using antiseptic 145C, and then cultured it in 37°C for 14 h to make curd, which is so-called bulk starter culture. The concentration of viable cells is 3×10^9 CFU/mL.

Then sterilize the raw milk by the above antiseptic in 95°C for 20 min and cool to 4°C, add the *Lactobacillus rhamnosus* CCFM1107 bulk starter culture to make the concentration larger than 10^6 CFU/mL, and keep it in 4°C. So the milk containing *Lactobacillus rhamnosus* CCFM1107 is obtained.

Applied Example 2: Using *Lactobacillus rhamnosus* CCFM1107 to make milk powder

Frist of all, make bulk starter culture of *Lactobacillus rhamnosus* CCFM1107.

The original *Lactobacillus rhamnosus* CCFM1107 strain is inoculated in skimmed milk with sterilization with a ratio of 5% based on the weight of said MRS liquid medium, and then activated in 37°C for 12 h. And then the activated culture is continued for two generations under the same condition.

The activated culture material is inoculated in MRS liquid medium with a ratio of 4% based on the volume of said MRS liquid medium, and then cultured in 37°C for 16 h and centrifuged in 4°C for 15 min with 4000r/min. Remove the supernatant and suspense the cell precipitation by sterilized skim milk. The so-called bulk starter culture with the concentration of viable bacteria of 1×10^9 CFU/mL is obtained.

Then raw milk is sterilized under 140°C for 2 s by PT-20C-R tube-plate combined ultrahigh temperature (UHT) fungicide, made by Japan Powerpoint International Ltd.. Cool the raw milk to 37°C, and the bulk starter culture of *Lactobacillus rhamnosus* CCFM1107 is inoculated in the raw milk with a ratio of 4% based on the volume of said MRS liquid medium. After culturing for 16 h in 37°C, the fermented milk containing *Lactobacillus rhamnosus* CCFM1107 is obtained. Add the fermented milk of *Lactobacillus rhamnosus* CCFM1107 into sterilized raw milk as the volume ratio 1:3. Use the type of GYB40-10S high pressure homogenizer sold by Shanghai Donghua high pressure homogenizer factory to homogenize the mixture and use the vacuum concentration cauldron sold by Yangzhou City Food Machinery Factory to concentrate it in vacuum. Then use the experimental spray dryer sold by Shanghai Wodi Technical Ltd. to spray dry it in order to get the milk powder containing *Lactobacillus rhamnosus* CCFM1107.

Applied Example 3: Using *Lactobacillus rhamnosus* CCFM1107 to make milk capsule Products

Frist of all, make bulk starter culture of *Lactobacillus rhamnosus* CCFM1107.

The strain *Lactobacillus rhamnosus* CCFM1107 is inoculated in MRS liquid medium with a ratio of 3% based on the weight of said MRS liquid medium, and then activated in 37°C for 16 h. And the activated culture is continued for two generations under the same condition.

The activated culture material is inoculated in MRS liquid medium with a ratio of 2% based on the volume of said MRS liquid medium, and then cultured in 37°C for 18 h and centrifuged in 4°C for 15 min with 4000r/min. Remove the supernatant and suspense the cell precipitation by sterilized skim milk. The so-called bulk starter culture with the concentration of viable bacteria of 2×10^9 CFU/mL is obtained.

Then raw milk is sterilized under 140°C for 2 s by PT-20C-R tube-plate combined ultra high temperature (UHT) fungicide, made by Japan Powerpoint International Ltd.. Cool the raw milk to 37°C, and the bulk starter culture of *Lactobacillus rhamnosus* CCFM1107 is inoculated in raw milk with a ratio of 4% based on the volume of said MRS liquid medium. After culturing for 16 h in 37°C, the fermented milk containing *Lactobacillus rhamnosus* CCFM1107 is obtained. Add the fermented milk of *Lactobacillus rhamnosus* CCFM1107 into sterilized raw milk as the volume ratio 1:3. Use the type of GYB40-10S high pressure homogenizer sold by Shanghai Donghua high pressure homogenizer factory to homogenize the mixture and use the vacuum concentration cauldron sold by Yangzhou City Food Machinery Factory to concentrate it in vacuum. Then use the experimental spray dryer sold by Shanghai Wodi Technical Ltd. to spray dry it in order to get the milk powder which can be put into capsules and made capsule products.

Applied Example 4: Using *Lactobacillus rhamnosus* CCFM1107 to make fermented milk

Frist of all, make bulk starter culture of *Lactobacillus rhamnosus* CCFM1107. The

original *Lactobacillus rhamnosus* CCFM1107 strain is inoculated in skim milk sterilized in 110°C for 10 min using 145C antiseptic sold by SPX/APV company with a ratio of 12% based on the weight of said MRS liquid medium. Then cultured it in 37°C for 16 h till the skimmed milk begin to curd. And then the activated culture is continued for two generations under the same condition. The fermented skimmed milk obtained is as the mother starter culture.

The mother starter culture, 3% volume of skim milk, is inoculated in skim milk, which has been sterilized in 110°C for 10 min using antiseptic 145C, and then cultured it in 37°C for 16 h to make curd, which is so-called bulk starter culture. The concentration of viable cells is 1×10^9 CFU/mL.

Then sterilize the raw milk by the above antiseptic in 95°C for 20 min and cool to 37°C, add the *Lactobacillus rhamnosus* CCFM1107 bulk starter culture, 4% volume of raw milk, as well as commercial starter cultures, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, 4% volume of raw milk. Then the inoculated milk is fermented in 37°C till the titratable acidity reaches to 0.6% (counted as lactic acid). And then the fermented milk is cooled to 4°C for cold storage, and then the fermented milk which comprises *Lactobacillus rhamnosus* CCFM1107 is obtained.

What is claimed is:

1. *Lactobacillus rhamnosus* CCFM1107, a bacterial strain which was deposited in China General Microbiological Culture Collection Center located in No.1 yard, Beichen West Road, Chaoyang district, Beijing on November 29, 2011 with the access number of CGMCC 5496.
2. An application of said *Lactobacillus rhamnosus* CCFM1107 according to claim 1 in preparing dairy product using bulk starter culture of *Lactobacillus rhamnosus* CCFM1107.
3. The application according to claim 2, characterized in that said dairy product is milk, milk powder, milk capsule products or fermented milk which comprise *Lactobacillus rhamnosus* CCFM1107.
4. The application according to claim 2, characterized in that said bulk starter culture of *Lactobacillus rhamnosus* is prepared according to the following preparation method:

the *Lactobacillus rhamnosus* CCFM1107 strain is inoculated in skimmed milk with sterilization under the temperature of 110 °C for 10 min with a ratio of 12% based on the weight of said skimmed milk, then the inoculated skimmed milk is cultured under the temperature of 37 °C for 14-16 h till the skimmed milk begin to curd, and then the activated culture is continued for two generations under the same condition, and then the fermented skimmed milk obtained is as the mother starter culture;

said mother starter culture is inoculated in new skimmed milk with sterilization under the temperature of 110 °C for 10 min with a ratio of 3-5% based on the weight of said new skimmed milk, then the inoculated new skimmed milk is cultured under the temperature of 37 °C for 14-16 h till the new skimmed milk begin to curd, then said bulk starter culture with a concentration of viable bacterium of $1-3 \times 10^9$ CFU/mL is obtained.

5. The application according to claim 2, characterized in that said bulk starter culture of *Lactobacillus rhamnosus* is prepared according to the following preparation method:

the *Lactobacillus rhamnosus* CCFM1107 strain is inoculated in MRS liquid medium with a ratio of 1-5% based on the weight of said MRS liquid medium, then the inoculated medium is cultured under the temperature of 37 °C for 12-16 h for activation, and the activated culture is continued for two generations under the same condition, and then the activated culture material is inoculated in new MRS liquid medium with a ratio of 2-4% based on the volume of said new MRS liquid medium, then the new inoculated medium is cultured under the temperature of 37 °C for 16-18 h, then the medium is centrifuged with speed of 4000 r/min and temperature of 4 °C for 15 min, and then the supernatant is removed, then the cell sediment obtained is suspended using sterilized skimmed milk, and then said bulk starter culture with a

concentration of viable bacterium of $1-3 \times 10^9$ CFU/mL is obtained.

6. The application according to claim 3, characterized in that said milk which comprise *Lactobacillus rhamnosus* CCFM1107 is prepared according to the following procedure:

raw milk is sterilized via pasteurization with the temperature of 95 °C for 20 min or via high temperature sterilization with the temperature of 140 °C for 2 s and then is cooled to 4 °C, and then said bulk starter culture of *Lactobacillus rhamnosus* CCFM1107 according to claim 4 or 5 is added to make the concentration of bacterium reaches more than 10^6 CFU/mL and then is kept in 4 °C for cold storage, and then the milk which comprise *Lactobacillus rhamnosus* CCFM1107 is obtained.

7. The application according to claim 3, characterized in that said milk powder or milk capsule product which comprise *Lactobacillus rhamnosus* CCFM1107 is prepared according to the following procedure:

raw milk is sterilized via pasteurization with the temperature of 95 °C for 20 min or via high temperature sterilization with the temperature of 140 °C for 2 s and then the sterilized milk is cooled to 37 °C, and then said bulk starter culture of *Lactobacillus rhamnosus* CCFM1107 according to claim 4 or 5 is inoculated in the sterilized milk with a ratio of 4% based on the volume of said sterilized milk, then the inoculated sterilized milk is fermented under the temperature of 37 °C for 16 h, and then the fermented milk which comprise *Lactobacillus rhamnosus* CCFM1107 is obtained; then, said fermented milk which comprise *Lactobacillus rhamnosus* CCFM1107 is inoculated in new sterilized milk with a ratio of 1:3 based on the volume of said new sterilized milk, and then the milk is processed via homogenization, vacuum concentration and spray drying to obtain milk powder which comprise *Lactobacillus rhamnosus* CCFM1107;

The said milk powder which comprise *Lactobacillus rhamnosus* CCFM1107 into enclosed capsule, and then milk capsule products is obtained.

8. The application according to claim 3, characterized in that said fermented milk which comprise *Lactobacillus rhamnosus* CCFM1107 is prepared according to the following procedure:

raw milk is sterilized via pasteurization with the temperature of 95 °C for 20 min or via high temperature sterilization with the temperature of 140 °C for 2 s and then the sterilized milk is cooled to 37 °C, and then a ratio of 3-5% of said bulk starter culture of *Lactobacillus rhamnosus* CCFM1107 according to claim 4 or 5 as well as a ratio of 3-5% of commercial starter cultures used for preparing fermented milk both based on the volume of said sterilized milk are inoculated in the sterilized milk, and then after being mixed uniformly, the inoculated milk is fermented under the temperature of 37 °C till the titration acidity reaches to 0.6-0.7% calculated by lactic acid, and then the fermented milk is cooled to 4 °C for cold storage, and then the fermented milk which comprise *Lactobacillus rhamnosus* CCFM1107 is obtained.

9. The application according to claim 8, characterized in that said commercial starter cultures are *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

10. The application according to any one of claim 6, 7 and 8, characterized in that said raw milk is one or more kinds of raw milk selected from skimmed milk, fresh milk and reconstituted milk, said milk refers to cow milk, goat milk or horse milk.