

**FERMENTED SUPERNATANT OF LACTIC ACID BACTERIA FOR USE
AS A MEDICAMENT FOR IMPROVING SYMPTOMS OF SARCOPENIA,
OBESITY AND SARCOPENIC OBESITY**

BACKGROUND

Field of Invention

[0001] The present invention relates to a fermented supernatant of lactic acid bacteria for use as a medicament for improving symptoms of sarcopenia. More particularly, the present invention relates to a fermented supernatant of lactic acid bacteria for use as a medicament for increasing FNDC5 gene expression, muscle mass and/or decreasing fat mass, thereby improving symptoms of sarcopenia, obesity and/or sarcopenic obesity.

Description of Related Art

[0002] Preventing and/or delaying disabilities and/or chronic diseases become an important issue as getting older. Sarcopenia and/or obesity are the main causes of disabilities and/or chronic diseases in older adults. The characteristic symptoms of sarcopenia include generalized and systematic loss of skeletal muscle mass and functions. A patient may experience a decreasing physical activity and a worse quality of life if his/her sarcopenia becomes worse, and the patient is at higher risk of diseases, disabilities, falls and even death.

[0003] Obesity is a medical condition with an abnormal or excessive accumulation of fat, in which the excessive fat easily results in insulin resistance and/or chronic inflammation, leading to many chronic diseases, such as metabolic syndromes, cardiovascular disease and/or diabetes. Moreover, obesity is positively related

to musculoskeletal disorders (e.g., degenerative arthritis). The medical condition with increased fat mass coupled with reduced muscle mass is called sarcopenic obesity, in which compared to patients with pure obesity (also called peripheral obesity, i.e., a condition of increased fat mass without reducing muscle mass), patients with sarcopenic obesity not only have a lower motor ability but also are at increased risks of chronic diseases. However, sarcopenic obesity is hardly improved by calorie control, since reduced calorie intake may not only cause lower fat mass but also result in lower muscle mass.

[0004] Therefore, increasing muscle mass and/or decreasing fat mass are basic principles for improving sarcopenia and/or obesity, in which the principles can include but not limited to a healthy diet and exercise, and the healthy diet replaces carbohydrates with protein to the benefit of muscle synthesis. Moreover, besides burning fat effectively, exercising can also cause the secretion of irisin, which can promote hypertrophy of skeletal muscles and/or browning of white adipocytes, thereby forming beige fat that can metabolite fatty acid. However, few studies have focused on methods of increasing irisin.

[0005] In view of the above, it is necessary to provide a fermented supernatant of lactic acid bacteria for use as a medicament for improving symptoms of sarcopenia, obesity and/or sarcopenic obesity.

SUMMARY

[0006] Therefore, an aspect of the present invention provides a fermented supernatant of lactic acid bacteria for use as a medicament for improving symptoms of sarcopenia, in which the medicament comprises the fermented supernatant of the lactic acid bacteria as an active ingredient, thereby increasing

a transcription activity of FNDC5 promoter. The fermented supernatant of the lactic acid bacteria can be obtained by performing a fermentation step on the lactic acid bacteria, followed by a solid-liquid separation step and a sterilization step, for example. The lactic acid bacteria can be selected from the group consisting of *Lactobacillus rhamnosus* GKLC1, *Lactobacillus acidophilus* GKA7, *Lactobacillus casei* GKC1 and any combination thereof, for example.

[0007] Another aspect of the present invention provides a fermented supernatant of lactic acid bacteria for use as a medicament for improving symptoms of obesity, in which the medicament can include an effective dose of the aforementioned fermented supernatant of the lactic acid bacteria as an active ingredient, thereby promoting transcription of FNDC5 promoter.

[0008] The other aspect of the present invention provides a cell-free fermented product of lactic acid bacteria for use as a medicament for improving symptoms of sarcopenic obesity, in which the medicament can include an effective dose of the aforementioned cell-free fermented product of the lactic acid bacteria as an active ingredient, thereby up-regulating transcription of FNDC5 promoter.

[0009] According to the aforementioned aspect of the present invention, a fermented supernatant of lactic acid bacteria for use as a medicament for improving symptoms of sarcopenia is provided. The medicament comprises the fermented supernatant of the lactic acid bacteria as an active ingredient, thereby increasing the transcription activity of FNDC5 promoter, in which the fermented supernatant of the lactic acid bacteria can be obtained by performing a fermentation step at 32°C to 42°C on the lactic acid bacteria, followed by a solid-liquid separation step and a sterilization step, and the lactic acid bacteria can be selected from the group consisting of *Lactobacillus rhamnosus* GKLC1,

Lactobacillus acidophilus GKA7, *Lactobacillus casei* GKC1 and any combination thereof. The aforementioned *Lactobacillus rhamnosus* GKLC1 is deposited in China General Microbiological Culture Collection Center (CGMCC) on January 12th, 2018 under an accession number of CGMCC No. 15202. The aforementioned *Lactobacillus acidophilus* GKA7 is deposited in National Institute of Technology and Evaluation (NITE), Patent Microorganisms Depositary on August 5th, 2022 under an accession number of NITE BP-03701. The aforementioned *Lactobacillus casei* GKC1 is deposited in CGMCC on January 12th, 2018 under an accession number of CGMCC No. 15204.

[0010] In one embodiment of the present invention, the fermentation step is performed with a lactic acid bacterial culture medium. Based on 100 wt% of the lactic acid bacterial culture medium, the lactic acid bacterial culture medium can include 1 weight (wt) % to 10 wt% of carbon sources, 1 wt% to 10 wt% of nitrogen sources and water with the balance.

[0011] In one embodiment of the present invention, the carbon sources can be selected from the group consisting of glucose, sucrose, lactose, fructose, mannose, sorbitol, glycerin, molasses and any combination thereof, for example.

[0012] In one embodiment of the present invention, the nitrogen sources can be selected from the group consisting of soy protein, yeast extract, beef extract, casein powder, whey protein powder, fish protein hydrolysate, plant-extracted protein, peptone and any combination thereof, for example.

[0013] In one embodiment of the present invention, an effective dose of the fermented supernatant of the lactic acid bacteria to a cell *in vitro* can be 50 µg/mL to 200 µg/mL, for example.

[0014] In one embodiment of the present invention, the effective dose of the fermented supernatant of the lactic acid bacteria to a cell *in vitro* can be 50 µg/mL to 100 µg/mL, for example, when the lactic acid bacteria is the *Lactobacillus rhamnosus* GKLC1.

[0015] In one embodiment of the present invention, the effective dose of the fermented supernatant of the lactic acid bacteria to a cell *in vitro* can be 100 µg/mL to 200 µg/mL, for example, when the lactic acid bacteria is the *Lactobacillus casei* GKC1 and/or the *Lactobacillus acidophilus* GKA7.

[0016] According to the aforementioned aspect of the present invention, a fermented supernatant of lactic acid bacteria for use as a medicament for improving symptoms of obesity is provided. The medicament can include an effective dose of the fermented supernatant of the lactic acid bacteria as an active ingredient, in which the fermented supernatant of the lactic acid bacteria can be obtained by performing a fermentation step at 32°C to 42°C on the lactic acid bacteria, followed by a solid-liquid separation step and a sterilization step. The lactic acid bacteria can be selected from the group consisting of *Lactobacillus rhamnosus* GKLC1, *Lactobacillus acidophilus* GKA7, *Lactobacillus casei* GKC1 and any combination thereof, thereby promoting transcription of FNDC5 promoter. An accession number of *Lactobacillus rhamnosus* GKLC1 is CGMCC No. 15202. An accession number of *Lactobacillus acidophilus* GKA7 is NITE BP-03701. An accession number of *Lactobacillus casei* GKC1 is CGMCC No. 15204. The effective dose of the fermented supernatant of the lactic acid bacteria to a cell *in vitro* can be 50 µg/mL to 200 µg/mL, for example.

[0017] In one embodiment of the present invention, the effective dose of the fermented supernatant of the lactic acid bacteria to the cell *in vitro* can be 50

μg/mL to 100 μg/mL, for example, when the lactic acid bacteria is the *Lactobacillus rhamnosus* GKLC1.

[0018] In one embodiment of the present invention, the effective dose of the fermented supernatant of the lactic acid bacteria to the cell *in vitro* can be 100 μg/mL to 200 μg/mL, for example, when the lactic acid bacteria is the *Lactobacillus casei* GKC1 and/or the *Lactobacillus acidophilus* GKA7.

[0019] In one embodiment of the present invention, the fermentation step is performed with a lactic acid bacterial culture medium, and based on 100 wt% of the lactic acid bacterial culture medium, the lactic acid bacterial culture medium can include 1 wt% to 10 wt% of carbon sources, 1 wt% to 10 wt% of nitrogen sources and water with the balance.

[0020] In one embodiment of the present invention, the carbon sources can be selected from the group consisting of glucose, sucrose, lactose, fructose, mannose, sorbitol, glycerin, molasses and any combination thereof, for example.

[0021] In one embodiment of the present invention, the nitrogen sources can be selected from the group consisting of soy protein, yeast extract, beef extract, casein powder, whey protein powder, fish protein hydrolysate, plant-extracted protein, peptone and any combination thereof, for example.

[0022] According to the other aspect of the present invention, a cell-free fermented product of lactic acid bacteria for use as a medicament for improving symptoms of sarcopenic obesity is provided. The medicament includes the cell-free fermented product of the lactic acid bacteria as an active ingredient, thereby up-regulating transcription of FNDC5 promoter. The cell-free fermented product of the lactic acid bacteria can be obtained by performing a fermentation step at 32°C to 42°C on the lactic acid bacteria with a lactic acid bacterial culture

medium, followed by a solid-liquid separation step and a sterilization step. The lactic acid bacteria can be selected from the group consisting of *Lactobacillus rhamnosus* GKLC1, *Lactobacillus acidophilus* GKA7, *Lactobacillus casei* GKC1 and any combination thereof, for example. An accession number of *Lactobacillus rhamnosus* GKLC1 is CGMCC No. 15202. An accession number of *Lactobacillus acidophilus* GKA7 is NITE BP-03701. An accession number of *Lactobacillus casei* GKC1 is CGMCC No. 15204.

[0023] In one embodiment of the present invention, based on 100 wt% of the lactic acid bacterial culture medium, the lactic acid bacterial culture medium can include 1 wt% to 10 wt% of carbon sources, 1 wt% to 10 wt% of nitrogen sources and water with the balance.

[0024] In one embodiment of the present invention, the carbon sources can be selected from the group consisting of glucose, sucrose, lactose, fructose, mannose, sorbitol, glycerin, molasses and any combination thereof, for example.

[0025] In one embodiment of the present invention, the nitrogen sources can be selected from the group consisting of soy protein, yeast extract, beef extract, casein powder, whey protein powder, fish protein hydrolysate, plant-extracted protein, peptone and any combination thereof, for example.

[0026] In one embodiment of the present invention, an effective dose of the cell-free fermented product of the lactic acid bacteria to a cell *in vitro* can be 50 µg/mL to 200 µg/mL, for example.

[0027] In one embodiment of the present invention, the effective dose of the cell-free fermented product of the lactic acid bacteria to the cell *in vitro* can be 50 µg/mL to 100 µg/mL, for example, when the lactic acid bacteria is the *Lactobacillus rhamnosus* GKLC1.

[0028] In one embodiment of the present invention, the effective dose of the cell-free fermented product of the lactic acid bacteria to the cell *in vitro* can be 100 µg/mL to 200 µg/mL, for example, when the lactic acid bacteria is the *Lactobacillus casei* GKC1 and/or the *Lactobacillus acidophilus* GKA7.

[0029] By applying the fermented supernatant of the lactic acid bacteria for use as a medicament for improving symptoms of sarcopenia, obesity and/or sarcopenic obesity, in which the fermented supernatant is obtained by subjecting the specific strains of lactic acid bacteria with the fermentation step, the solid-liquid separation step and sterilization step. Experiments have proved that the fermented supernatant of the lactic acid bacteria can increase the transcription activity of the FNDC5 promotor *in vitro*, thereby promoting hypertrophy of skeletal muscles and/or browning of white adipocytes. Thus, the fermented supernatant of the lactic acid bacteria can be used to prepare an oral composition of increasing FNDC5 gene expression, increasing muscle mass and decreasing fat mass.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] The invention can be more fully understood by reading the following detailed description of the embodiment, with reference made to the accompanying drawings as follows.

[0031] FIGs. 1A to 1B showed a visible light microscopy image (FIG. 1A) and a fluorescent microscopy image (FIG. 1B), respectively, according to an embodiment of the present invention.

[0032] FIGs. 2A to 2D showed visible light microscopy images (FIGs. 2A, 2C) and fluorescent microscopy images (FIGs. 2B, 2D) of the transfected cells treated

with 50 µg/mL (FIGs. 2A, 2B) and 100 µg/mL (FIGs. 2C, 2D) of the GKLC1 lyophilized powder for 24 hours, respectively, according to an embodiment of the present invention.

[0033] FIGs. 3A to 3D showed visible light microscopy images (FIGs. 3A, 3C) and fluorescent microscopy images (FIGs. 3B, 3D) of the transfected cells treated with 100 µg/mL (FIGs. 2A, 2B) and 200 µg/mL (FIGs. 2C, 2D) of the GKA7 lyophilized powder for 24 hours, respectively, according to an embodiment of the present invention.

[0034] FIGs. 4A to 4D showed visible light microscopy images (FIGs. 4A, 4C) and fluorescent microscopy images (FIGs. 4B, 4D) of the transfected cells treated with 100 µg/mL (FIGs. 4A, 4B) and 200 µg/mL (FIGs. 4C, 4D) of the GKC1 lyophilized powder for 24 hours, respectively, according to an embodiment of the present invention.

DETAILED DESCRIPTION

[0035] As mentioned above, the present invention provides a fermented supernatant of lactic acid bacteria for use as a medicament for improving symptoms of sarcopenia, obesity and sarcopenic obesity, in which the medicament includes the fermented supernatant of the lactic acid bacteria as an active ingredient, thereby increasing fibronectin type III domain-containing protein (FNDC) 5 gene expression, muscle mass and decreasing fat mass.

[0036] The “fermented supernatant of the lactic acid bacteria” mentioned herein refers to the cell-free fermented product of the lactic acid bacteria obtained by subjecting the lactic acid bacteria to a fermentation step, followed by a solid-liquid separation step and a sterilization step. In one embodiment, the fermented

supernatant of the lactic acid bacteria is the single active ingredient of the medicament. The “lactic acid bacteria” mentioned herein refer to the bacteria that can produce lactic acid and/or acetic acid after breaking down sugar such as galactose, glucose, sucrose and fructose, etc. In one embodiment, the lactic acid bacteria can be selected from the group consisting of *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus casei* and any combination thereof, for example.

[0037] In one embodiment, the *Lactobacillus rhamnosus* can be *Lactobacillus rhamnosus* GKLC1 deposited in China General Microbiological Culture Collection Center (CGMCC) (Address of depositary institution: Institute of Microbiology, Chinese Academy of Sciences, No. 1 Beichen West Road, Chaoyang District, Beijing 100101, China) on January 12th, 2018 under an accession number of CGMCC No. 15202, for example. In one embodiment, the *Lactobacillus acidophilus* can be *Lactobacillus acidophilus* GKA7 deposited in National Institute of Technology and Evaluation (NITE), Patent Microorganisms Depositary (NPMD) (Address of depositary institution: #122, 2-5-8 Kazusakamatari Kisarazu-shi Chiba 292-0818, Japan) on August 5th, 2022 under an accession number of NITE BP-03701, for example. In one embodiment, the *Lactobacillus casei* can be *Lactobacillus casei* GKC1, deposited in CGMCC on January 12th, 2018 under an accession number of CGMCC No. 15204. The *Lactobacillus rhamnosus* GKLC1, the *Lactobacillus acidophilus* GKA7 and the *Lactobacillus casei* GKC1 are also deposited in Bioresource Collection and Research Center (BCRC) at the Food Industry Development and Development Institute (FIRC) (Address of depositary institution: No. 331 on Shih-Pin Road, Hsinchu, Taiwan), in which the *Lactobacillus rhamnosus* GKLC1 is deposited under an accession number of BCRC 910828 on February, 12th, 2018, the *Lactobacillus acidophilus* GKA7 is

deposited under an accession number of BCRC 910985 on April, 10th, 2020, and the *Lactobacillus casei* GKC1 is deposited under with an accession number of BCRC 910825 on February, 12th, 2018. The deposits are made under the terms of the Budapest Treaty.

[0038] In one embodiment, the lactic acid bacteria can consist of the *Lactobacillus rhamnosus*, the *Lactobacillus acidophilus* and the *Lactobacillus casei*. In one embodiment, a cell ratio of the *Lactobacillus rhamnosus*, the *Lactobacillus acidophilus* and the *Lactobacillus casei* can be 1~5:1~5:1~5, for example.

[0039] The “fermentation step” mentioned herein refers to a step that cultures the lactic acid bacteria with a lactic acid bacterial culture medium, thereby obtaining the fermented product of the lactic acid bacteria. The kind of lactic acid bacterial culture medium is not specially limited as long as it can fulfill the nutritional requirement for growing the lactic acid bacteria, and the kind of lactic acid bacterial culture medium can be a commercially available culture medium. In one embodiment, the lactic acid bacterial culture medium can include but not limited to carbon sources and nitrogen sources, in which the kinds of the carbon sources and the nitrogen sources are not specially limited as long as they can be used by the lactic acid bacteria. In some specific embodiment, the carbon sources can be selected from the group consisting of glucose, sucrose, lactose, fructose, mannose, sorbitol, glycerin, molasses and any combination thereof, for example. In some specific embodiment, the nitrogen sources can be selected from the group consisting of soy protein, yeast extract, beef extract, casein powder, whey protein powder, fish protein hydrolysate, plant-extracted protein, peptone and any combination thereof, for example.

[0040] The amounts of the carbon sources and the nitrogen sources of the lactic acid bacterial culture medium are not specially limited as long as the contents fulfill the nutritional requirement for the growth and/or the metabolism of the lactic acid bacteria. In one embodiment, based on 100 wt% of the lactic acid bacterial culture medium, the lactic acid bacterial culture medium can include but not limited to 1 wt% to 10 wt% of the carbon sources, 1 wt% to 10 wt% of the nitrogen sources and water with the balance. In a better embodiment, based on 100 wt% of the lactic acid bacterial culture medium, the amount of the carbon sources of the lactic acid bacterial culture medium can be 2 wt% to 7 wt%, and the amount of the nitrogen sources of the lactic acid bacterial culture medium can be 2 wt% to 7 wt%, for example. In one specific embodiment, the lactic acid bacterial culture medium consists of 5 wt% of glucose, 5 wt% of yeast extract and water with the balance. In another specific embodiment, the lactic acid bacterial culture medium consists of 2 wt% of glucose, 1 wt% of peptone, 1 wt% of beef extract, 0.5 wt% of yeast extract and water with the balance.

[0041] The temperature and time of the fermentation step are not specially limited as long as the fermented product of the lactic acid bacteria can be obtained. In some embodiments, the temperature of the fermentation step is 32°C to 42°C and/or the time of the fermentation step is 12 hours to 18 hours, so as to increase the activities of the lactic acid bacteria, thereby obtaining the fermented supernatant of the lactic acid bacteria with the better effects of increasing FNDC5 gene expression, muscle mass and/or decreasing fat mass.

[0042] The “solid-liquid separation step” mentioned herein refers to a step that separates the solid portion and the liquid portion of the fermented product of the lactic acid bacteria. The solid portion of the fermented product of the lactic acid

bacteria can include but not limited to the viable cells of the lactic acid bacteria and/or other materials that are not water-soluble, and the liquid portion of the fermented product of the lactic acid bacteria, which is also called fermented supernatant of the lactic acid bacteria, can include but not limited to the material secreted by the lactic acid bacteria during the fermentation step. The method of the solid-liquid separation step is not specially limited and can be a centrifugation step and/or a filtration step. In one specific embodiment, the centrifugation step can be performed at 2000 revolutions per minute (rpm) to 12000 rpm for 2 minutes to 15 minutes, for example, be performed at 5000 rpm) to 10000 rpm for 5 minutes to 15 minutes.

[0043] The “sterilization step” mentioned herein refers to a step that makes the lactic acid bacteria remaining in the fermented supernatant thereof lose the activities of growth, metabolism and/or die with a chemical method and/or a physical method. It should be noticed that to ensure the safety of the oral composition, the sterilization step is better performed with the physical method. In one embodiment, the physical method of the sterilization step can be a heat-inactivated treatment, for example. In one specific embodiment, the heat inactive treatment can be performed at 120°C to 130°C for 0.5 minutes to 3 minutes, for example, 121°C for 1 minute, so that the lactic acid bacteria remaining in the fermented supernatant thereof can lose their activities, but the effects of the fermented supernatant of the lactic acid bacteria to increase the transcription activity of FNDC5 promoter can be kept.

[0044] After being subjected to a sterilization step, the fermented supernatant of the lactic acid bacteria can be selectively subjected to a drying treatment. The method of the drying treatment is not specially limited and can be performed with

a conventional method, such as a lyophilization treatment, a vacuum drying treatment or a spray drying treatment. The product obtained by the method is the cell-free fermented product of the lactic acid bacteria.

[0045] The *in vitro* cell experiment proves that the fermented supernatant of the lactic acid bacteria has the effects of increasing FNDC5 gene expression, muscle mass and/or decreasing fat mass, thereby having the potential to improve sarcopenia, obesity and/or sarcopenic, obesity.

[0046] To “increase FNDC5 gene expression” mentioned herein refers to increasing the RNA amount and/or the protein amount of the FNDC5 gene. The method to increase FNDC5 gene expression is not specially limited and can be to increase the transcription activity of FNDC5 promoter, to promote and/or to up-regulate the transcription of FNDC5 promoter, to elongate the RNA degradation rate of FNDC5 and/or to increase the translation activity of the RNA of FNDC5.

[0047] It should be noted that the transcription activity of the FNDC5 promoter can be evaluated by the expression amount of the reporter gene that is operably linked to the FNDC5 promoter (i.e., located downstream of the FNDC5 promoter). In one embodiment, the reporter gene can be green fluorescent protein (GFP), β -galactosidase gene and/or luciferase.

[0048] To “increase muscle mass” mentioned herein refers to increasing numbers of muscle cells, muscle mass and/or the cross-sectional area of muscle. The phenomenon of increasing muscle mass and/or the cross-sectional area of muscle without increasing the number of muscle cells is also called hypertrophy. The aforementioned FNDC5 is an irisin precursor. The study has found that irisin can promote the myoblast fusion of the skeletal muscle, activate the satellite

cells, increase protein synthesis and/or decrease protein degradation, thereby promoting the hypertrophy of skeletal muscles.

[0049] To “decrease fat mass” mentioned herein refers to consuming the fat in the adipocytes. In detail, the adipocytes can be categorized into white adipocytes, brown adipocytes and beige adipocytes. Each of the white adipocytes has a single big oil droplet (i.e., unicular) and a small number of mitochondria, in which the big oil droplet can store the excess heat in the human body into fat. Compared to the white adipocytes, brown adipocytes have smaller volumes, more mitochondria and more separated oil droplets, in which the brown adipocytes can express genes such as uncoupling protein (UCP) 1, so as to metabolite the fat stored in the small oil droplets.

[0050] The beige adipocytes have cell morphologies and function midway between the white adipocytes and the brown adipocytes. Specifically, the number of mitochondria of a beige adipocyte is more than that of a white adipocyte but smaller than that of a brown adipocyte. Moreover, the volume of an oil droplet of a beige adipocyte is smaller than that of a white adipocyte and bigger than that of a brown adipocyte. When not stimulated, the function of a beige adipocyte is similar to a white adipocyte. However, when stimulated by exterior stimulations (e.g., low temperature, adrenaline stimulants and/or para-adrenergic stimulants) and thus activated, the function of a beige adipocyte is similar to a brown adipocyte, in which the function includes UCP1 gene expression and thus fat metabolism.

[0051] After the browning of white adipocytes, the beige adipocytes can be formed, in which the “browning” mentioned herein refers to the transformation of the white adipocytes into the beige adipocytes and/or the process of changing

the cell function from fat storage into fat metabolite after the beige adipocytes are activated by exterior stimulations. Studies have found that irisin can promote the browning of white adipocytes, thereby increasing the numbers of the beige adipocytes and/or the activated beige adipocytes. Therefore, the effect to decrease fat mass can be reached.

[0052] In one embodiment, the effective dose of the fermented supernatant of the lactic acid bacteria to a cell *in vitro* can be 50 μ L to 200 μ L, for example, 50 μ L to 150 μ L, or 80 μ L to 120 μ L, or else 100 μ L. In one specific embodiment, the effective dose of the fermented supernatant of the lactic acid bacteria to a cell *in vitro* is 50 μ g/mL to 100 μ g/mL when the fermented supernatant of the lactic acid bacteria is obtained by the *Lactobacillus rhamnosus* GKLC1. In one specific embodiment, the effective dose of the fermented supernatant of the lactic acid bacteria to a cell *in vitro* is 100 μ g/mL to 200 μ g/mL when the fermented supernatant of the lactic acid bacteria is obtained by the *Lactobacillus casei* GKC1 and/or the *Lactobacillus acidophilus* GKA7.

[0053] In addition, when being applied, the aforementioned medicament can be an oral composition, and the oral composition can be a food composition or a medical composition, for example. In one embodiment, the oral composition can selectively include food or pharmaceutical-acceptable carrier, excipient, diluter, auxiliary, preservative, filler and/or additive. In one embodiment, the oral composition consists of the fermented supernatant of the lactic acid bacteria and the carrier.

[0054] It will be apparent to those skilled in the art that various modifications and variations can be made to the structure of the present invention without departing from the scope or spirit of the invention. In view of the foregoing, it is intended

that the present invention cover modifications and variations of this invention provided they fall within the scope of the followed claims.

Embodiment 1. Source of strain

[0055] *Lactobacillus rhamnosus* GKLC1 and *Lactobacillus casei* GKC1 were bought from CGMCC, and *Lactobacillus acidophilus* GKA7 was bought from NITE, in which an accession number of *Lactobacillus rhamnosus* GKLC1 was CGMCC No. 15202, an accession number of *Lactobacillus acidophilus* GKA7 was NITE BP-03701, and an accession number of *Lactobacillus casei* GKC1 was CGMCC No. 15204. Please refer to the Taiwan Patent TW I715177 B (application no. TW108131434A) for the microorganism traits and the culturing method of the *Lactobacillus rhamnosus* GKLC1, and refer to the Taiwan Patent TW I754929 B (application no. TW109115097A) for the microorganism traits and the culturing method of the *Lactobacillus acidophilus* GKA7 and the *Lactobacillus casei* GKC1. The patents were cited as a reference herein. Hereafter the *Lactobacillus rhamnosus* GKLC1 was referred to as strain GKLC1, the *Lactobacillus acidophilus* GKA7 was referred to as strain GKA7, and the *Lactobacillus casei* GKC1 was referred to as strain GKC1.

[0056] Additionally, the aforementioned *Lactobacillus acidophilus* GKA7 was separated from human breast milk, and the 16S rRNA gene sequence of *Lactobacillus acidophilus* GKA7 included the nucleic acid fragment as shown in SEQ ID NO: 1. *Lactobacillus casei* GKC1 was separated from human baby facets, and the 16S rRNA gene sequence of *Lactobacillus casei* GKC1 included the nucleic acid fragment as shown in SEQ ID NO: 2.

Embodiment 2. Preparation of lactic acid bacteria lyophilized powder

[0057] The strain GKLC1 was subjected to a fermentation step at 37°C with a lactic acid bacterial culture medium for 16 hours, thereby obtaining a GKLC1 fermented product, in which the lactic acid bacterial culture medium consisted of 2 wt% glucose, 1 wt% peptone, 1 wt% beef extract, 0.5 wt% yeast extract and water with the balance.

[0058] Then, the GKLC1 fermented product was subjected to a centrifuge step operated at 5,000 rpm to 10,000 rpm for 5 minutes to 12 minutes, thereby obtaining a GKLC1 fermented supernatant. After that, the GKLC1 fermented supernatant was subjected to a sterilization step at 121°C for 1 minute to obtain a sterilized GKLC1 fermented product. Subsequently, the sterilized GKLC1 fermented product was subjected to a lyophilization treatment, thereby obtaining GKLC1 lyophilized powder.

[0059] GKA7 lyophilized powder and GKC1 lyophilized powder were made by the same method except that the GKA7 lyophilized powder was made by the strain GKA7, and the GKC1 lyophilized powder was made by the strain GKC1.

Embodiment 3. Evaluation of lyophilized powder of fermented supernatant of lactic acid bacteria to increase effect of increasing transcription activity of FNDC5 promoter

1. Building a cell model

[0060] The transformed cells transformed with fluorescent plasmids were inoculated in a lysogeny broth (LB) culture medium containing 0.1 volume % of Kanamycin, followed by a shaking culture at 37°C for 10 hours, so as to obtain a transformation cell culture product. After being collected in a centrifuge tube, the transformation cell culture product was subjected to centrifugation at a speed of 6000 g at 4°C for 15 minutes, followed by the removal of the supernatant,

thereby obtaining a pellet. Then, the pellet was subjected to a plasmid DNA extraction by a commercially available plasmid DNA extraction kit (Geneaid™ Midi plasmid kit, catalog number: Cat. #PI025), thereby obtaining fluorescent plasmids. The fluorescent plasmids included a mouse FNDC5 promoter and a fluorescent protein-expressing gene, and the fluorescent protein-expressing gene was operably linked to FNDC5 promoter.

[0061] The human embryonic kidney (HEK) 293 cells (accession number: ATCC CRL-1573, hereafter referred to as HEK293 cells) were incubated in a 24-well cell culture plate with Dulbecco's modified Eagle medium (DMEM), in which there were 5×10^4 HEK293 cells in each well. Then, the 24-well cell culture plate was placed in an incubator to incubate HEK293 cells in 5% CO₂ at 37°C for 1 day.

[0062] 50 µL of plasmid solution and 50 µL of transfection reagent solution were prepared, in which the plasmid solution included 1 µg fluorescent plasmid and the balance of serum-free culture medium, and the transfection reagent solution included 2 µL of a commercially available transfection reagent (Lipofectamine 2000 reagent, Thermofisher) and the balance of serum-free culture medium. After the plasmid solution and the transfection reagent solution were mixed, the mixed product was stood at room temperature for 20 minutes, thereby obtaining a transfection solution.

[0063] Then, the DMEM that had cultured HEK293 cells in the 24-well cell culture medium plate was replaced with 300 µL of serum-free DMEM, followed by the addition of 100 µL of the transfection reagent, and then the 24-well cell culture medium plate was placed into the cell incubator to incubate HEK293 cells in 5% CO₂ at 37°C for 4 hours to 6 hours, thereby obtaining transfected cells.

2. Evaluating the green fluorescent intensity of the transfected cells treated with the lyophilized powder of the fermented supernatant of the lactic acid bacteria

[0064] The transfected cells were incubated with DMEM at 37°C for 24 hours. The aforementioned transfected cells were separated into a control group and an experimental group, in which the transfected cells of the control group were not treated with the lyophilized powder of the fermented supernatant of the lactic acid bacteria (i.e., the GKCL1 lyophilized powder, the GKA7 lyophilized powder and the GKC1 lyophilized powder), and the transfected cells of the experimental group were treated with 50 µg/mL, 100 µg/mL and 200 µg/mL of the GKCL1 lyophilized powder, the GKA7 lyophilized powder and the GKC1 lyophilized powder. Then, the visible light and fluorescence (excited light was the blue light of light wavelength 488 nm) microscopy images were observed and recorded under a fluorescence microscope. The green light produced by transfected cells indicated an increase in the transcription activity of the FNDC5 promoter that was operably linked by the green fluorescent protein, so the transfected cells expressed the green fluorescent protein. A higher green fluorescent intensity indicated a higher transcription activity of the FNDC5 promoter.

[0065] Please refer to FIGs. 1A, 1B, 2A to 2D, 3A to 3D and 4A to 4D. FIGs. 1A to 1B showed a visible light microscopy image (FIG. 1A) and a fluorescent microscopy image (FIG. 1B), respectively, according to an embodiment of the present invention. FIGs. 2A to 2D showed visible light microscopy images (FIGs. 2A, 2C) and fluorescent microscopy images (FIGs. 2B, 2D) of transfected cells treated with 50 µg/mL (FIGs. 2A, 2B) and 100 µg/mL (FIGs. 2C, 2D) of the GKLC1 lyophilized powder for 24 hours, respectively, according to an embodiment of the present invention. FIGs. 3A to 3D showed visible light microscopy images (FIGs.

3A, 3C) and fluorescent microscopy images (FIGs. 3B, 3D) of the transfected cells treated with 100 $\mu\text{g/mL}$ (FIGs. 2A, 2B) and 200 $\mu\text{g/mL}$ (FIGs. 2C, 2D) of the GKA7 lyophilized powder for 24 hours, respectively, according to an embodiment of the present invention. FIGs. 4A to 4D showed visible light microscopy images (FIGs. 4A, 4C) and fluorescent microscopy images (FIGs. 4B, 4D) of the transfected cells treated with 100 $\mu\text{g/mL}$ (FIGs. 4A, 4B) and 200 $\mu\text{g/mL}$ (FIGs. 4C, 4D) of the GKC1 lyophilized powder for 24 hours, respectively, according to an embodiment of the present invention. Area 201, area 301 and area 401 defined by dashed lines indicated the locations of cells showed in the corresponding visible light microscopy images. Area 203, area 303 and area 403 defined by dashed lines indicated the areas having no cells at the observed layers. Area 205, area 305 and area 405 defined by dashed lines indicated noise signals.

[0066] As shown in FIGs. 1A and 1B, the transfected cells of the control group were not treated with the lyophilized powder of the fermented supernatant of the lactic acid bacteria and the transfected cells of the control group did not emit green fluorescence, and therefore FIG. 1B showed no green fluorescent signals. As shown in FIGs. 2B and 2D, the cells in the areas 201 showed green fluorescent signals compared to FIG. 1A, indicating that after being treated with 50 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ of the GKLC1 lyophilized powder for 24 hours, respectively, the transfected cells could emit green fluorescent signals. Notably, the green fluorescent intensity of the transfected cells treated with 100 $\mu\text{g/mL}$ of the GKLC1 lyophilized powder was higher than that of the transfected cells treated with 50 $\mu\text{g/mL}$ of the GKLC1 lyophilized powder.

[0067] As shown in FIGs. 3B and 3D, the cells in the areas 301 showed green fluorescent signals compared to FIG. 1A, indicating that after being treated with 100 $\mu\text{g/mL}$ and 200 $\mu\text{g/mL}$ of the GKA7 lyophilized powder for 24 hours, respectively, the transfected cells could emit green fluorescent signals. Notably, the green fluorescent intensity of the transfected cells treated with 200 $\mu\text{g/mL}$ of the GKA7 lyophilized powder was higher than that of the transfected cells treated with 100 $\mu\text{g/mL}$ of the GKA7 lyophilized powder.

[0068] As shown in FIGs. 4B and 4D, the cells in the areas 401 showed green fluorescent signals compared to FIG. 1A, indicating that after being treated with 100 $\mu\text{g/mL}$ and 200 $\mu\text{g/mL}$ of the GKC1 lyophilized powder for 24 hours, respectively, the transfected cells could emit green fluorescent signals. Notably, the green fluorescent intensity of the transfected cells treated with 200 $\mu\text{g/mL}$ of the GKC1 lyophilized powder was higher than that of the transfected cells treated with 100 $\mu\text{g/mL}$ of the GKC1 lyophilized powder. It should be noted that the green fluorescent intensity was obtained by subtracting the background signal value from the fluorescent intensity of the cells. Moreover, whether a green fluorescent signals were the fluorescent expressed by the cells or noise signals should be determined by the location of the cells shown in the corresponding visible light microscopy images.

[0069] Notably, the fermented supernatant of the lactic acid bacteria obtained from the *Lactobacillus rhamnosus* GKLC1, the *Lactobacillus acidophilus* GKA7 or the *Lactobacillus casei* GKC1 could all increase the transcription activity of FNDC5 promoter, meaning that they had the same, similar or related pharmacological action, and had no antagonistic effect. Thus, the FNDC5 gene expression and the muscle mass could be increased and/or the fat mass could

be decreased no matter the fermented supernatants of the lactic acid bacteria obtained from the *Lactobacillus rhamnosus* GKLC1, the *Lactobacillus acidophilus* GKA7 or the *Lactobacillus casei* GKC1 were administered alone or in combination with each other.

[0070] In sum, the fermented supernatant of the lactic acid bacteria obtained by *Lactobacillus rhamnosus* GKLC1, *Lactobacillus acidophilus* GKA7 and *Lactobacillus casei* GKC1 can increase the transcription activity of FNDC5 promoter, thereby promoting the hypertrophy of skeletal muscles and/or browning of white adipocytes. Therefore, the fermented supernatant of the lactic acid bacteria of the present invention can be used to increase FNDC5 gene expression, muscle mass and/or to decrease fat mass, and has the potential to improve symptoms of sarcopenia, obesity and/or sarcopenic obesity.

[0071] Noting that although the specific strains of the lactic acid bacteria, the specific incubation method, the specific process, the specific methods of administration, the specific cell model and the specific evaluation methods to explain the fermented supernatant of the lactic acid bacteria for use as a medicament for improving symptoms of sarcopenia, obesity and sarcopenic obesity, it will be apparent to those skilled in the art that the present invention is not specially limited to what have mentioned. Without departing from the scope or spirit of the invention, it is intended that other strains of the lactic acid bacteria, other incubation methods, other processes, other methods of administration, other cell models and other evaluation methods can also explain the present invention.

[0072] Although the present invention has been described in considerable detail with reference to certain embodiments thereof, other embodiments are possible.

Therefore, the spirit and scope of the appended claims should not be limited to the description of the embodiments contained herein.

WHAT IS CLAIMED IS:

1. A fermented supernatant of lactic acid bacteria for use as a medicament for improving symptoms of sarcopenia, wherein the medicament comprises the fermented supernatant of the lactic acid bacteria as an active ingredient, thereby increasing a transcription activity of FNDC5 promoter, the fermented supernatant of the lactic acid bacteria is obtained by performing a fermentation step at 32°C to 42°C on the lactic acid bacteria, followed by a solid-liquid separation step and a sterilization step, the lactic acid bacteria is selected from the group consisting of *Lactobacillus rhamnosus* GKLC1, *Lactobacillus acidophilus* GKA7, *Lactobacillus casei* GKC1 and any combination thereof, the *Lactobacillus rhamnosus* GKLC1 is deposited in China General Microbiological Culture Collection Center (CGMCC) on January 12th, 2018 under an accession number of CGMCC No. 15202, the *Lactobacillus acidophilus* GKA7 is deposited in National Institute of Technology and Evaluation (NITE), Patent Microorganisms Depository on August 5th, 2022 under an accession number of NITE BP-03701, and the *Lactobacillus casei* GKC1 is deposited in CGMCC on January 12th, 2018 under an accession number of CGMCC No. 15204.

2. The fermented supernatant of the lactic acid bacteria for use as the medicament for improving symptoms of sarcopenia of claim 1, wherein the fermentation step is performed with a lactic acid bacterial culture medium, and based on 100 wt% of the lactic acid bacterial culture medium, the lactic acid bacterial culture medium comprises 1 weight (wt) % to 10 wt% of carbon sources, 1 wt% to 10 wt% of nitrogen sources and water with a balance.

3. The fermented supernatant of the lactic acid bacteria for use as the medicament for improving symptoms of sarcopenia of claim 2, wherein the carbon sources are selected from the group consisting of glucose, sucrose,

lactose, fructose, mannose, sorbitol, glycerin, molasses and any combination thereof.

4. The fermented supernatant of the lactic acid bacteria for use as the medicament for improving symptoms of sarcopenia of claim 2, wherein the nitrogen sources are selected from the group consisting of soy protein, yeast extract, beef extract, casein powder, whey protein powder, fish protein hydrolysate, plant-extracted protein, peptone and any combination thereof.

5. The fermented supernatant of the lactic acid bacteria for use as the medicament for improving symptoms of sarcopenia of claim 1, wherein an effective dose of the fermented supernatant of the lactic acid bacteria to a cell *in vitro* is 50 µg/mL to 200 µg/mL.

6. The fermented supernatant of the lactic acid bacteria for use as the medicament for improving symptoms of sarcopenia of claim 1, wherein an effective dose of the fermented supernatant of the lactic acid bacteria to a cell *in vitro* is 50 µg/mL to 100 µg/mL when the lactic acid bacteria is the *Lactobacillus rhamnosus* GKLC1.

7. The fermented supernatant of the lactic acid bacteria for use as the medicament for improving symptoms of sarcopenia of claim 1, wherein an effective dose of the fermented supernatant of the lactic acid bacteria to a cell *in vitro* is 100 µg/mL to 200 µg/mL when the lactic acid bacteria is the *Lactobacillus casei* GKC1 and/or the *Lactobacillus acidophilus* GKA7.

8. A fermented supernatant of lactic acid bacteria for use as a medicament for improving symptoms of obesity, wherein the medicament comprises an effective dose of the fermented supernatant of the lactic acid bacteria as an active ingredient, thereby promoting a transcription of FNDC5 promoter, the fermented supernatant of the lactic acid bacteria is obtained by performing a fermentation

step at 32°C to 42°C on the lactic acid bacteria, followed by a solid-liquid separation step and a sterilization step, the lactic acid bacteria is selected from the group consisting of *Lactobacillus rhamnosus* GKLC1, *Lactobacillus acidophilus* GKA7, *Lactobacillus casei* GKC1 and any combination thereof, an accession number of *Lactobacillus rhamnosus* GKLC1 is CGMCC No. 15202, an accession number of *Lactobacillus acidophilus* GKA7 is NITE BP-03701, an accession number of *Lactobacillus casei* GKC1 is CGMCC No. 15204, and the effective dose of the fermented supernatant of the lactic acid bacteria to a cell *in vitro* is 50 µg/mL to 200 µg/mL.

9. The fermented supernatant of the lactic acid bacteria for use as the medicament for improving symptoms of obesity of claim 8, wherein the effective dose of the fermented supernatant of the lactic acid bacteria to the cell *in vitro* is 50 µg/mL to 100 µg/mL when the lactic acid bacteria is the *Lactobacillus rhamnosus* GKLC1.

10. The fermented supernatant of the lactic acid bacteria for use as the medicament for improving symptoms of obesity of claim 8, wherein the effective dose of the fermented supernatant of the lactic acid bacteria to the cell *in vitro* is 100 µg/mL to 200 µg/mL when the lactic acid bacteria is the *Lactobacillus casei* GKC1 and/or the *Lactobacillus acidophilus* GKA7.

11. The fermented supernatant of the lactic acid bacteria for use as the medicament for improving symptoms of obesity of claim 8, wherein the fermentation step is performed with a lactic acid bacterial culture medium, and based on 100 wt% of the lactic acid bacterial culture medium, the lactic acid bacterial culture medium comprises 1 wt % to 10 wt% of carbon sources, 1 wt% to 10 wt% of nitrogen sources and water with a balance.

12. The fermented supernatant of the lactic acid bacteria for use as the medicament for improving symptoms of obesity of claim 11, wherein the carbon sources are selected from the group consisting of glucose, sucrose, lactose, fructose, mannose, sorbitol, glycerin, molasses and any combination thereof.

13. The fermented supernatant of the lactic acid bacteria for use as the medicament for improving symptoms of obesity of claim 11, wherein the nitrogen sources are selected from the group consisting of soy protein, yeast extract, beef extract, casein powder, whey protein powder, fish protein hydrolysate, plant-extracted protein, peptone and any combination thereof.

14. A cell-free fermented product of lactic acid bacteria for use as a medicament for improving symptoms of sarcopenic obesity, wherein the medicament comprises the cell-free fermented product of the lactic acid bacteria as an active ingredient, thereby up-regulating transcription of FNDC5 promoter, the cell-free fermented product of the lactic acid bacteria is obtained by performing a fermentation step at 32°C to 42°C on the lactic acid bacteria with a lactic acid bacterial culture medium, followed by a solid-liquid separation step and a sterilization step, the lactic acid bacteria is selected from the group consisting of *Lactobacillus rhamnosus* GKLC1, *Lactobacillus acidophilus* GKA7, *Lactobacillus casei* GKC1 and any combination thereof, an accession number of *Lactobacillus rhamnosus* GKLC1 is CGMCC No. 15202, an accession number of *Lactobacillus acidophilus* GKA7 is NITE BP-03701, and an accession number of *Lactobacillus casei* GKC1 is CGMCC No. 15204.

15. The cell-free fermented product of lactic acid bacteria for use as the medicament for improving symptoms of sarcopenic obesity of claim 14, wherein based on 100 wt% of the lactic acid bacterial culture medium, the lactic acid

bacterial culture medium comprises 1 wt% to 10 wt% of carbon sources, 1 wt% to 10 wt% of nitrogen sources and water with a balance.

16. The cell-free fermented product of lactic acid bacteria for use as the medicament for improving symptoms of sarcopenic obesity of claim 15, wherein the carbon sources are selected from the group consisting of glucose, sucrose, lactose, fructose, mannose, sorbitol, glycerin, molasses and any combination thereof.

17. The cell-free fermented product of lactic acid bacteria for use as the medicament for improving symptoms of sarcopenic obesity of claim 15, wherein the nitrogen sources are selected from the group consisting of soy protein, yeast extract, beef extract, casein powder, whey protein powder, fish protein hydrolysate, plant-extracted protein, peptone and any combination thereof.

18. The cell-free fermented product of lactic acid bacteria for use as the medicament for improving symptoms of sarcopenic obesity of claim 14, wherein an effective dose of the cell-free fermented product of the lactic acid bacteria to a cell *in vitro* is 50 µg/mL to 200 µg/mL.

19. The cell-free fermented product of lactic acid bacteria for use as the medicament for improving symptoms of sarcopenic obesity of claim 14, wherein an effective dose of the cell-free fermented product of the lactic acid bacteria to a cell *in vitro* is 50 µg/mL to 100 µg/mL when the lactic acid bacteria is the *Lactobacillus rhamnosus* GKLC1.

20. The cell-free fermented product of lactic acid bacteria for use as the medicament for improving symptoms of sarcopenic obesity of claim 14, wherein an effective dose of the cell-free fermented product of the lactic acid bacteria to a cell *in vitro* is 100 µg/mL to 200 µg/mL when the lactic acid bacteria is the *Lactobacillus casei* GKC1 and/or the *Lactobacillus acidophilus* GKA7.