USE OF *PEDIOCOCCUS PENTOSACEUS* GKP4 IN MANUFACTURE OF MEDICAMENT OF IMPROVING SARCOPENIA

BACKGROUND

Field of Invention

[0001] The present invention is related to a use of lactic acid bacteria in the manufacture of a medicament of improving sarcopenia. In particular, the present invention is related to a use of *Pediococcus pentosaceus* GKP4 in the manufacture of a medicament of improving sarcopenia.

Description of Related Art

[0002] Sarcopenia is a syndrome characterized by progressive and generalized loss of muscle mass and functions of skeletal muscles, followed by a decline in mobility and the quality of life of the patient as well as an increase in the risks of disability and falling. Furthermore, since the muscle is related to protein storage and/or blood glucose metabolism of human body, sarcopenia may also induce other chronic diseases (e.g., diabetes).

[0003] Sarcopenia can be subdivided into acute sarcopenia and chronic sarcopenia. The acute sarcopenia often occurs in hospitalized patients who are old and/or having acute diseases or acute injury since these patients experience muscle disuse. Muscle disuse may cause dysregulation of muscle homeostasis and results in a decrease amount of muscle growth and an increased amount of muscle loss. The acute sarcopenia not only increases the risks of postoperative inflammation and extends recovery period and/or

hospitalization period of the hospitalized patients, but may also cause acute loss of organ function, respiratory failure and/or death. In addition, the acute sarcopenia may also develop into chronic sarcopenia, thereby influencing the quality of life of the hospitalized patients after recovery.

[0004] Causes of sarcopenia can be divided into primary causes and secondary causes. The primary causes include muscle loss caused by aging. The secondary causes include diseases (such as organ failure, malignant tumor and osteoarthritis), less activities (such as long-time sitting, movement disorder or disability caused by diseases) and/or malnutrition (such as insufficient intake of nutrients, poor nutrient absorption, anorexia, overnutrition and/or obesity). At present, sarcopenia cannot be controlled with drugs and can only be relieved or improved by exercise, control of chronic diseases, improvement of inflammatory symptoms and/or supplement of specific nutrients.

[0005] Therefore, it is urgent to provide a use of *Pediococcus pentosaceus* GKP4 in the manufacture of a medicament to improve the sarcopenia.

SUMMARY

[0006] Therefore, one aspect of the present invention is to provide a use of *Pediococcus pentosaceus* GKP4 in the manufacture of a medicament of improving sarcopenia.

[0007] Another aspect of the present invention is to provide a use of *Pediococcus pentosaceus* GKP4 in the manufacture of a medicament of improving sarcopenia, in which the medicament can include but not limited to an effective dosage of *Pediococcus pentosaceus* GKP4 as an active ingredient,

and the effective dosage of the *Pediococcus pentosaceus* GKP4 for an adult human can be 240 mg/60 kg body weight (bw)/day to 4,800 mg/60 kg bw/day, for example.

[0008] The other aspect of the present invention is to provide a use of *Pediococcus pentosaceus* GKP4 in the manufacture of a medicament of improving sarcopenia, in which the medicament can include but not limited to an effective dosage of *Pediococcus pentosaceus* GKP4 as an active ingredient, and the effective dosage of the *Pediococcus pentosaceus* GKP4 for a mouse can be 50 mg/kg bw/day to 1,000 mg/kg bw/day, for example.

[0009] A further aspect of the present invention is to provide a use of *Pediococcus pentosaceus* GKP4 in the manufacture of a medicament of improving sarcopenia, in which the medicament can include but not limited to an effective dosage of a lyophilized powder of the *Pediococcus pentosaceus* GKP4 as an active ingredient, and the medicament is to be administered daily to a sarcopenia subject for at least 14 days.

[0010] According to the aforementioned aspects of the present invention, a use of *Pediococcus pentosaceus* GKP4 for preparing a medicament of improving sarcopenia is provided. The medicament can include but not limited to an effective dosage of the *Pediococcus pentosaceus* GKP4 as an active ingredient, and the medicament is to be administered daily to a subject for a period of time. The *Pediococcus pentosaceus* GKP4 can be deposited on November 6, 2020 at NITE Patent Microorganisms Depositary of National Institute of Technology and Evaluation (NITE) with an accession number NITE BP-03317, for example. In one embodiment of the present invention, the

period of time can be at least 14 days, for example. In one embodiment of the present invention, the *Pediococcus pentosaceus* GKP4 improves sarcopenia by relieving muscle loss.

[0011] According to another aspect of the present invention, the medicament can include but not limited to an effective dosage of *Pediococcus pentosaceus* GKP4 as an active ingredient, in which an accession number of *Pediococcus pentosaceus* GKP4 can be NITE BP-03317, and the effective dosage of the *Pediococcus pentosaceus* GKP4 for an adult human can be 240 mg/60 kg bw/day to 4,800 mg/60 kg bw/day/day, or example. In one embodiment of the present invention, the sarcopenia can be an acute sarcopenia, for example. According to the other aspect of the present invention, the medicament can include but not limited to an effective dosage of *Pediococcus pentosaceus* GKP4 as an active ingredient, in which an accession number of *Pediococcus pentosaceus* GKP4 can be NITE BP-03317, and the effective dosage of the *Pediococcus pentosaceus* GKP4 for a mouse can be 50 mg/kg bw/day to 1,000 mg/kg bw/day, for example. In one embodiment of the present invention, the sarcopenia can be an acute sarcopenia, for example.

[0012] According to a further aspect of the present invention, a use of *Pediococcus pentosaceus* GKP4 in the manufacture of a medicament of improving sarcopenia is provided. The medicament can include but not limited to an effective dosage of a lyophilized powder of the *Pediococcus pentosaceus* GKP4 as an active ingredient, and accession number of *Pediococcus pentosaceus* GKP4 is NITE BP-03317. The medicament is to be administered daily to a sarcopenia subject for at least 14 days.

[0013] In one embodiment of the present invention, *Pediococcus pentosaceus* GKP4 improves sarcopenia by relieving muscle loss of the sarcopenia subject. In one embodiment of the present invention, *Pediococcus pentosaceus* GKP4 improves sarcopenia by increasing a muscle strength of the sarcopenia subject. In one embodiment of the present invention, *Pediococcus pentosaceus* GKP4 improves sarcopenia by a muscle endurance of the sarcopenia subject.

[0014] By applying the use of *Pediococcus pentosaceus* GKP4 in the manufacture of a medicament of improving sarcopenia of the present invention, the *Pediococcus pentosaceus* GKP4 can improve sarcopenia by increasing the muscle mass, the muscle strength and/or the muscle endurance of the subject, and thus can improve sarcopenia.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] The invention can be more fully understood by reading the followed detailed description of the embodiment, with reference made to the accompanying drawings as follows:

[0016] FIG. 1 was a bar chart illustrating the relative weights of the soleus muscles of the hind limbs of the mice in different groups according to an embodiment of the present invention.

[0017] FIG. 2 was a bar chart illustrating the relative weights of the gastrocnemius muscles of the hind limbs of the mice in different groups according to an embodiment of the present invention.

[0018] FIG. 3 was a bar chart illustrating the grip strengths of the mice in different groups according to an embodiment of the present invention.

[0019] FIG. 4 was a bar chart illustrating the numbers of electric shocks on the mice in different groups according to an embodiment of the present invention.

DETAILED DESCRIPTION

[0020] Singular forms, “a”, “an”, and “the”, as mentioned in the present invention also cover plural references, unless otherwise stated in the context. A value range (such as 10% to 11% of A) includes its upper and lower limits (i.e., 10% ≤ A ≤ 11%) if not otherwise stated specially; the value range, for which if no lower limit is defined (such as B lower than 0.2%, or B below 0.2%), can have a lower limit of zero (i.e., 0% ≤ B ≤ 0.2%). The aforementioned terms are used for illustrating and helping understand the present invention rather than limiting the scope thereof.

[0021] As mentioned above, the present invention provides a use of *Pediococcus pentosaceus* GKP4 in the manufacture of a medicament of improving sarcopenia, in which the medicament can include but not limited to an effective dosage of the *Pediococcus pentosaceus* GKP4 as an active ingredient to relieve the muscle loss of a subject. The medicament containing *Pediococcus pentosaceus* GKP4 is referred to as GKP4 medicament herein.

[0022] In one embodiment, the *Pediococcus pentosaceus* GKP4 (also called strain GKP4) has been deposited on November 6, 2020 at NITE Patent Microorganisms Depositary of National Institute of Technology and Evaluation

(NITE) located at 122, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba 292-0818, Japan) with an accession number NITE BP-03317.

[0023] In one embodiment, the GKP4 medicament can include a lyophilized powder of *Pediococcus pentosaceus* GKP4, for example. In one embodiment, the lyophilized powder of *Pediococcus pentosaceus* GKP4 can be obtained by performing a fermentation culture step, a solid-liquid separation step, a drying step and a grinding step on the *Pediococcus pentosaceus* GKP4, for example. The fermentation culture step can be performed by culturing *Pediococcus pentosaceus* GKP4 in a culture solution for 15 hours to 24 hours at 35°C to 45°C, for example, in which the culture solution is a commercially available MRS culture solution having a pH value of 6.5±0.2, for example. The solid-liquid step separation can be performed by a known method such as a centrifugation treatment and/or a filtering step, for example. The drying step can be performed by a known water removal method, such as a lyophilization method, a vacuum drying method or a spray drying method. The grinding step can be performed by a known grinding method, such as a mechanical grinding method, a roll grinding method and a flowing-gas grinding method.

[0024] The dosage form of the GKP4 medicament is not specifically limited as long as it contains *Pediococcus pentosaceus* GKP4. In one embodiment, the dosage form of the GKP4 medicament can be an aqueous solution, a suspension, dispersion, an emulsion (single-phase or multiphase disperse system, unilamellar or multilamellar liposome), a hydrogel, a gel, a solid lipid nanoparticle, a tablet, a granule, a powder and/or a capsule and the like, for example. In one embodiment, the GKP4 medicament can selectively include

food or pharmaceutically acceptable carriers, excipients, diluents, adjuvants and/or additives, which can be a solvent, an emulsifying agent, a suspending agent, a disintegrating agent, a binding agent, a stabilizing agent, a chelating agent, a diluent, a gelling agent, a preservative, a lubricant and/or an absorption retardant and the like, for example.

[0025] No limitation is on the administration amount and administration times of the GKP4 medicament as long as the effective dosage of *Pediococcus pentosaceus* GKP4 is reached. In one embodiment, the effective dosage of *Pediococcus pentosaceus* GKP4 for a mouse can be 50 mg/kg bw/day to 1,000 mg/kg bw/day, for example. In a specific example, the effective dosage of *Pediococcus pentosaceus* GKP4 for a mouse is 500 mg/kg bw/day. In one embodiment, the effective dosage of *Pediococcus pentosaceus* GKP4 for an adult human can be 240 mg/60 kg bw/day to 4,800 mg/60 kg bw/day/day, for example. If the effective dosage of *Pediococcus pentosaceus* GKP4 is lower than the aforementioned range, the GKP4 medicament could hardly improve sarcopenia. However, if the effective dosage of *Pediococcus pentosaceus* GKP4 is higher than the aforementioned range, the effect of the medicament on improving sarcopenia can not get better, but the manufacturing cost of the medicament will increase.

[0026] The conversion of the effective dosage for mouse and adult human can be estimated according to an estimation method for initial trials (Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers) announced by the Food and Drug Administration on 2005, in which 12.3-fold of the recommended intake amount per kilogram body weight

for human equals to 1-fold of the dose for mouse. In one embodiment, the medicament can improve sarcopenia as the GKP4 medicament is administered daily to the subject for at least 14 days, such as 14 days to 30 days. In one embodiment, the administration timing of the GKP4 medicament is not limited, and can be once per day, for example.

[0027] The “sarcopenia” can refer to the definition of European Working Group on Sarcopenia in Older People 2 (EWGSOP2) on 2018, in which the indicators of sarcopenia include the muscle strength (such as grip strength), the muscle mass (such as the skeletal muscle mass of four limbs) and physical performance (such as 400-meter walking test) of a subject. When the muscle mass and physical performance of the subject are up to standard, but the muscle strength is below standard, the subject is determined to suffer from “probable sarcopenia”. When the physical performance of the subject is up to standard, but the muscle strength and muscle mass are below standard, the subject is determined to suffer from “sarcopenia”. When the muscle strength, muscle mass and physical performance of the subject are below standard, the subject is determined to suffer from “severe sarcopenia”. Moreover, according to the definition of EWGSOP2, if the muscle strength and/or muscle mass of the subject decrease dramatically within 6 months, the subject is determined to suffer from acute sarcopenia, or otherwise chronic sarcopenia.

[0028] Animal experiments have proven that a mouse subjected with a cast-immobilization treatment can be deemed as a sarcopenia mouse. In details, after a mouse is subjected with a cast-immobilization treatment on the hind limbs for 1 week (herein referred to as cast-immobilization treated mouse),

the muscle masses of the soleus muscles and gastrocnemius muscles of the hind limbs as well as a significantly reduced grip strength of the fore limbs of the mouse significantly reduced, indicating that the mouse suffers from immobilization-induced acute muscle atrophy. Thus, the cast-immobilization treated mouse can be used as an animal model for acute sarcopenia. In addition, in treadmill trials, the cast-immobilization treated mouse is subjected to more electric shocks, proving that cast-immobilization treated mouse may also have a reduced muscle endurance resulting in a decreased physical performance. Thus, the cast-immobilization treated mouse can be used as an animal model for severe sarcopenia.

[0029] The aforementioned “improving sarcopenia” means that the muscle loss of the subject can be relieved. In other words, “improving sarcopenia” means that the muscle mass, muscle strength and muscle endurance of the subject increase. Animal experiments have proven that the GKP4 medicament can improve sarcopenia. In details, compared to the sarcopenia mice not administered with the GKP4 medicament, the sarcopenia mice administered with the GKP4 medicament have a 20% to 25% increase in the soleus muscles and a 5% to 10% increase in the gastrocnemius muscles of the hind limbs. In addition, the mice with sarcopenia administered with the GKP4 medicament has a 15% to 20% increase in the grip strength of the fore limbs. Moreover, in treadmill trials, the sarcopenia mouse administered with the GKP4 medicament receives 80% to 85% fewer electric shocks. In sum, the GKP4 medicament is proven to improve sarcopenia.

[0030] Several examples are used below to describe the application of the present invention, but are not intended to limit the present invention. Those of common knowledge in the technical field of the present invention can make various changes and modifications without departing from the spirit and scope of the present invention.

EXAMPLE 1. Strain source and the preparation of GKP4 lyophilized powder

[0031] The strain GKP4 used in the present invention was separated from the naturally fermented pickles of sour chili commercially available in Hualian, Taiwan, in which the following showed the separation method: the pickles of sour chili were diluted and homogenized to obtain a homogeneous liquid mixture. Then, the homogeneous liquid mixture was inoculated into an Man Rogosa and Sharp (MRS) culture solution and incubated at 37°C for 16 hours to screen out strains (including lactic acid bacteria, LAB) preferring to grow in a weak acid (pH 6.5±0.2) environment, thereby obtaining a LAB culture solution. Then, the LAB culture solution was diluted and respectively spread on 5 solid-state media (including: MRS medium agar, bidobacterium iodoacetate medium-25 (BIM-25) agar, agar containing potato starch and a yeast extract, bromocresol green MRS medium agar and bromocresol purple MRS medium agar) followed by an incubation at 37°C for 40 hours to obtain multiple single colonies, in which one of the single colonies was strain GKP4.

[0032] The DNA of the strain GKP4 was purified and subjected to a gene sequencing to obtain the nucleic acid fragment of the 16s rRNA gene of the strain GKP4 with a sequence shown in SEQ ID NO: 1. After sequence

alignment, the strain GKP4 was identified as *Pediococcus pentosaceus*. The DNA purification, gene sequencing and sequence alignment were performed by known methods and could be arbitrarily adjusted without affecting the following evaluation of the effect of the strain GKP4 in improving sarcopenia, and thus would not be not elaborate.

[0033] The *Pediococcus pentosaceus* strain GKP4 was deposited on November 6, 2020 at NITE Patent Microorganisms Depositary of National Institute of Technology and Evaluation (NITE) with an accession number NITE BP-03317.

[0034] The strain GKP4 was inoculated into an MRS culture solution and subjected to a fermentation step at 37°C for 16 hours to obtain a ferment product. Then, the ferment product was centrifuged at a speed of 10,000 rpm to obtain a bacterial pellet. Next, the bacterial pellet was mixed with 20 weight% of skim milk powder, followed by lyophilization to obtain GKP4 lyophilized powder. The lyophilization was performed with a known method and could be arbitrarily adjusted without affecting the following evaluation, and thus would not be not elaborate.

EXAMPLE 2. Establishment of an animal model of muscle atrophy.

[0035] An animal model of muscle atrophy was established by using C57BL/6 mice (herein referred to as mice), in which the mice were 4 weeks old and weighed 24 g to 26 g. The trial was in accordance with the 3R (replacement, reduction and refinement) principles of the Animal Ethics Committee. The mice were housed in an animal room, in which the temperature of the animal room was controlled at 22±2°C, the humidity was controlled at 55-65%, and a

light period was set from 7:00 to 19:00 every day while the dark period was set from 19:00 to next 7:00.

[0036] At first, the mice were housed in the animal room for 1 week to adapt to the environment of the animal room, a grip strength meter (GSM, Cat. No.: 47200; Manufacturer: Ugo Basile, Gemonio, Italy) and a treadmill (Name: animal treadmill; Cat. No.: 47300; Manufacturer: Ugo Basile). Next, the mice were randomly divided into 3 groups: an experimental group, a control group and a blank group with 5 mice per group.

[0037] The mice in the experimental group and control group were subjected with a cast-immobilization treatment for 7 days to induce muscle atrophy. Then, the cast was removed so that the mice in the experimental group and the control group were allowed to freely move around for 7 days. The mice in the blank group were allowed to move freely move around for 14 days. In the cast-immobilization treatment period and the free moving period (14 days in total), the mice in the experimental group were gavaged with the GKP4 composition every day, in which the GKP4 composition was an aqueous solution containing the GKP4 lyophilized powder. It was worth noting that the concentration of the GKP4 composition was properly adjusted to gavaged 500 mg/kg·bw/day of GKP4 lyophilized powder to mice in the experimental group, while the volume of the GKP4 composition was limited to a maximum of 10 mL/kg·bw/day.

[0038] Then, a grip strength trial and a treadmill trial were performed with the grip strength meter and the treadmill to evaluate the muscle strength and muscle endurance of the mice. Next, the mice were sacrificed, and the body

weights of the mice as well as the weights of the soleus muscles and gastrocnemius muscles of the hind limbs of the mice were measured to evaluate the muscle mass of the mice.

EXAMPLE 3. Evaluation of the effect of the GKP4 composition on the muscle mass of mouse.

[0039] A relative weight of the soleus muscles was obtained by calculating the ratio of the weight of the soleus muscles of the mouse hind limbs of a mouse to the body weight of the mouse. FIG. 1 was a bar chart illustrating the relative weights of the soleus muscles of the hind limbs of the mice in different groups according to an embodiment of the present invention, in which the horizontal axis represented different groups and the longitudinal axis represented the relative weight of the soleus muscles (unit: mg/g·bw). As shown in FIG. 1, the relative weight of the soleus muscles of the hind limbs of the mice in the control group is lower than that of the mice in the blank group, proving that the cast-immobilization treatment was able to simulate immobilization-induced muscle atrophy. However, the relative weight of the soleus muscles of the hind limbs of the mice in the experimental group was 0.06 mg higher than those in the control group (equivalent to a 23% increase), proving that the muscle mass of the cast-immobilization treated mouse was able to increase after administrated daily with the GKP4 composition for 14 days.

[0040] Next, a relative weight of the gastrocnemius muscles was obtained by calculating the ratio of the weight of the gastrocnemius muscles of the hind limbs of the mouse to the body weight of the mouse. FIG. 2 was a bar chart illustrating the relative weights of the gastrocnemius muscles of the hind limbs

of the mice in different groups according to an embodiment of the present invention, in which the horizontal axis represented different groups, the longitudinal axis represented the relative weight of the gastrocnemius muscles (unit: mg/g·bw), and the figure symbol "*" signified a statistically significant difference ($p<0.05$) comparing the control group tested by Student's t-test. As shown in FIG. 2, the relative weight of the gastrocnemius muscles of the hind limbs of the mice in the control group was lower than that of the mice in the blank group, proving that the cast-immobilization treatment could simulate the immobilization-induced muscle atrophy. However, the relative weight of the gastrocnemius muscles of the hind limbs of the mice in the experimental group was significantly higher than that of the mice in the control group (0.35 mg higher, equivalent to a 7% increase), proving that the muscle mass of the cast-immobilization treated mouse could increase after a administrated daily with the GKP4 composition for 14 days.

EXAMPLE 4. Evaluation of the effect of the GKP4 composition on the muscle strength of mouse.

[0041] A grip strength trial was performed by the grip strength meter to measure the grip strength of the fore limbs of a mouse and was operated as follows: the fore limbs of the mouse were made to grip a pull ring, and the tail end of the mouse was caught by a hand and pulled backward along a horizontal direction until the fore limbs of mouse released the pull ring. The grip strength was measured for three consecutive times, and the maximum was taken for statistics.

[0042] FIG. 3 was a bar chart illustrating the grip strengths of the mice in different groups according to an embodiment of the present invention, in which the horizontal axis showed the group, the longitudinal axis showed the grip strength (unit: gram-force, gf), and the figure symbol "*" signified a statistically significant difference ($p<0.05$) tested by Student's t-test. As shown in FIG. 3, the grip strength of the mice in the control group was reduced compared with that of the mice in the blank group, proving that the cast-immobilization treatment was able to simulate the immobilization-induced muscle atrophy. However, the grip strength of the mice in the experimental group was significantly higher than in the control group (16.8 gf, equivalent to a 17% increase), proving that the muscle strength of the cast-immobilization treated mouse was able to increase after a daily administration of the GKP4 composition.

Example 5. Evaluation of the effect of the GKP4 composition on the muscle endurance of mouse.

[0043] The muscle endurance of a mouse was measured by a treadmill trial and was operated as follows: the mouse was put on a treadmill having an uphill inclined conveyor belt conveying downward at a rate of 18 m/min to 20 m/min. Since an electric shock sensing device was mounted at the bottom of the treadmill, the electric shock sensing device would give an electric shock to the mouse when the mouse touched the electric shock sensing device. Generally speaking, the mouse would climb upward to avoid the electric shock. However, if the muscle endurance of the mouse was insufficient to overcome the speed of

the conveyor belt, the mouse would be conveyed to the bottom of the treadmill and subjected to an electric shock.

[0044] FIG. 4 was a bar chart illustrating the numbers of electric shocks of the mice in different groups according to an embodiment of the present invention, in which the horizontal axis showed the group, the longitudinal axis showed the numbers of electric shocks, and the figure symbol "*" signified a statistically significant difference ($p<0.05$) from the control group tested by Student's t-test. As shown in FIG. 4, the mice in the control group received more electrical shocks than the mice in the blank group did, proving that the cast-immobilization treatment was able to simulate the immobilization-induced muscle atrophy. However, the mice in the experimental group received fewer electric shocks than the mice in the control group did (453 fewer, equivalent to an 82.5% decrease), proving that the muscle endurance of the cast-immobilization treated mouse was able to increase after a daily administration of the GKP4 composition.

[0045] As shown above, the cast-immobilization treated mice had a reduced relative weight of the soleus muscles and/or gastrocnemius muscles (FIG. 1 and FIG. 2), reduced grip strength (FIG. 3) and received more electric shocks in the treadmill trial (FIG. 4). However, the cast-immobilization treated mice administered with the GKP4 composition had an increased relative weight of the soleus muscles and/or gastrocnemius muscles (FIG. 1 and FIG. 2), increased grip strength (FIG. 3) and received less electric shocks in the treadmill trial (FIG. 4) compared to the cast-immobilization treated mice not administered with the GKP4 composition. The results proved that the GKP4 composition was able to

relieve the muscle loss caused by the immobilization-induced muscle atrophy, indicating that the GKP4 composition was able to improve sarcopenia, in which the sarcopenia could be acute sarcopenia.

[0046] In conclusion, the aforementioned specific dosage forms, specific manufacturing processes, specific doses, specific administration modes, specific animal models and specific evaluation methods are only intended to illustrate and describe the use of *Pediococcus pentosaceus* GKP4 in the manufacture of a medicament of improving sarcopenia. However, the person skilled in the art should understand that the present invention is not limited to what have mentioned, and other dose forms, other manufacturing processes, other doses, other administration modes, other animal models and other evaluation methods can also be used for describing the use of *Pediococcus pentosaceus* GKP4 in the manufacture of a medicament of improving sarcopenia of the present invention without departing from the spirit and scope of the present invention. For example, other dosage forms, other manufacturing processes, other administration modes, other animal models and other evaluation methods can be used on the premise of not influencing the effect of *Pediococcus pentosaceus* GKP4 on improving sarcopenia.

[0047] 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. Use of *Pediococcus pentosaceus* GKP4 in the manufacture of a medicament of improving sarcopenia, wherein the medicament comprises an effective dosage of the *Pediococcus pentosaceus* GKP4 as an active ingredient, the medicament is to be administered daily to a subject for at least 14 days, and the *Pediococcus pentosaceus* GKP4 is deposited on November 6, 2020 at NITE Patent Microorganisms Depositary of National Institute of Technology and Evaluation (NITE) with an accession number NITE BP-03317.

2. The use according to claim 1, wherein the sarcopenia is an acute sarcopenia.

3. The use according to claim 1, wherein the *Pediococcus pentosaceus* GKP4 improves the sarcopenia by relieving muscle loss.

4. Use of *Pediococcus pentosaceus* GKP4 in the manufacture of a medicament of improving sarcopenia, wherein the medicament comprises an effective dosage of the *Pediococcus pentosaceus* GKP4 as an active ingredient, an accession number of *Pediococcus pentosaceus* GKP4 is NITE BP-03317, the medicament is to be administered daily to a subject for at least 14 days, and the effective dosage of the *Pediococcus pentosaceus* GKP4 for an adult human is 240 mg/60 kg body weight (bw)/day to 4,800 mg/60 kg bw/day.

5. The use according to claim 4, wherein the sarcopenia is an acute sarcopenia.

6. Use of *Pediococcus pentosaceus* GKP4 in the manufacture of a medicament of improving sarcopenia, wherein the medicament comprises an effective dosage of the *Pediococcus pentosaceus* GKP4 as an active ingredient, an accession number of *Pediococcus pentosaceus* GKP4 is NITE BP-03317,

the medicament is to be administered daily to a subject for at least 14 days, and the effective dosage of the *Pediococcus pentosaceus* GKP4 for a mouse is 50 mg/kg bw/day to 1,000 mg/kg bw/day.

7. The use according to claim 6, wherein the sarcopenia is acute sarcopenia.

8. Use of *Pediococcus pentosaceus* GKP4 in the manufacture of a medicament of improving sarcopenia, wherein the medicament comprises an effective dosage of a lyophilized powder of the *Pediococcus pentosaceus* GKP4 as an active ingredient, an accession number of *Pediococcus pentosaceus* GKP4 is NITE BP-03317, and the medicament is to be administered daily to a sarcopenia subject for at least 14 days.

9. The use according to claim 8, wherein the *Pediococcus pentosaceus* GKP4 improves the sarcopenia by relieving a muscle loss of the sarcopenia subject.

10. The use according to claim 8, wherein the *Pediococcus pentosaceus* GKP4 improves the sarcopenia by increasing a muscle strength of the sarcopenia subject.

11. The use according to claim 8, wherein the *Pediococcus pentosaceus* GKP4 improves the sarcopenia by increasing a muscle endurance of the sarcopenia subject.