

BIFIDOBACTERIUM BREVE, COMPOSITION COMPRISING
BIFIDOBACTERIUM BREVE OR CULTURE THEREOF,
AND USE OF *BIFIDOBACTERIUM BREVE* OR CULTURE
THEREOF

5

TECHNICAL FIELD

The present disclosure relates to a probiotic and its application, and in particular it relates to a *Bifidobacterium breve*, a composition containing *Bifidobacterium breve* or a
10 culture thereof, and the use of *Bifidobacterium breve* or a culture thereof.

BACKGROUND TECHNOLOGIES

Overweight, high blood glucose, high blood lipids, etc.
15 are all the main risk factors for cardiovascular disease while healthy diet and moderate exercise are one of the ways to avoid these risk factors.

It is currently known that glucagon-like peptide-1 (GLP-1) belonging to a type of incretin, has the effects of
20 inhibiting gastric motility, delaying gastric emptying, suppressing appetite, promoting insulin secretion from pancreatic islet β -cells, and inhibiting pancreatic glucagon secretion from pancreatic islet α -cells, and it is a hot target

for research on appetite regulation, blood sugar modulation, and weight control.

Probiotics are now widely accepted as a health food ingredient. Probiotics are defined as active microorganisms
5 that are present in sufficient amounts to alter the gastrointestinal flora of the host and produce beneficial health effects, wherein bacteria belonging to the genus *Lactobacillus* or belonging to genus *Bifidobacterium* are the most widely used. Probiotics can usually cause a decrease
10 in intestinal pH by lactic acid stimulation, which in turn produces products that contribute to the growth of more beneficial microorganisms and enhance the host's immune response to pathogens.

As mentioned above, a healthy diet is one of the ways
15 to avoid cardiovascular disease risk factors such as overweight, high blood glucose, and high blood lipids, and thus there is still an urgent need to conduct relevant research on probiotics as health foods in appetite regulation, blood glucose modulation, weight control, etc.,
20 to achieve effective health management through the consumption of probiotics.

SUMMARY OF THE INVENTION

The present disclosure provides a novel *Bifidobacterium breve* which is deposited in the Bioresource Collection and Research Center of Food Industry Research and Development Institute, and the deposit number of which is
5 BCRC 911145.

The present disclosure also provides a use of the foregoing novel *Bifidobacterium breve* or a culture thereof in the manufacture of a composition for reducing appetite and/or promoting glucagon-like peptide-1 (GLP-1)
10 secretion.

The present disclosure further provides a use of the foregoing novel *Bifidobacterium breve* or a culture thereof in the manufacture of a composition for inhibiting adipocyte maturation.

15 The present disclosure additionally provides a use of the novel above-mentioned *Bifidobacterium breve* or a culture thereof in the manufacture of a composition for reducing fat accumulation and/or promoting fat metabolism.

Moreover, the present disclosure provides a use of the
20 above-mentioned novel *Bifidobacterium breve* or a culture thereof in the manufacture of a composition for reducing body weight.

The present disclosure further provides a use of the

novel *Bifidobacterium breve* mentioned above or a culture thereof in the manufacture of a composition for modulating blood lipids.

The present disclosure provides a use of the novel
5 *Bifidobacterium breve* mentioned above or a culture thereof in the manufacture of a composition for modulating blood glucose.

In addition, the present disclosure further provides a probiotic composition, comprising the aforementioned
10 novel *Bifidobacterium breve* or a culture thereof; and a prebiotic.

In order to make the foregoing and other purposes, features, and advantages of the present disclosure more obvious and easier to understand, the following preferred
15 embodiments, together with the accompanying drawings, are described in detail as follows:

DESCRIPTION OF THE DRAWINGS

FIG. 1A shows the relative amount of glucagon-like
20 peptide-1 (GLP-1) secreted into the medium by the cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the

medium for culturing the cells is 0.25%).

FIG. 1B shows the relative expression of PYY gene in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 48 hours, the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.5%). Compared to the Mock group, **: $p < 0.01$.

FIG. 2A shows the relative amount of glucagon-like peptide-1 (GLP-1) secreted into the medium by the cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 (the final concentration of the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.125%). Compared to the Mock group, *: $p < 0.05$.

FIG. 2B shows the relative expression of PYY gene in cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 24 hours, the final concentration of the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.125%). Compared to the Mock group, ***: $p < 0.001$.

FIG. 3A shows the relative expression of C/EBPA gene in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 6 hours, the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.25%). Compared to the Mock group, ***: $p < 0.001$.

FIG. 3B shows the Oil red (lipid) content in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145. Compared to the Mock group, ***: $p < 0.001$.

FIG. 4A shows the relative expressions of UCP1 gene and UCP2 gene in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 24 hours, the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.125%). Compared to the Mock group, **: $p < 0.01$, ***: $p < 0.001$.

FIG. 4B shows the amount of glycerol in the cultured medium of cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for

culturing the cells is 0.25%). Compared to the Mock group,
*: $p < 0.05$.

FIG. 5 show the relative expressions of UCP1 gene and
UCP2 gene in cells treated with the dead bacteria
5 suspension of *Bifidobacterium breve* BCRC 911145 (cells
are cultured for 24 hours, the final concentration of the
dead bacteria suspension of *Bifidobacterium breve* BCRC
911145 in the medium for culturing the cells is 0.25%).
Compared to the Mock group, ***: $p < 0.001$.

10 FIG. 6A shows the relative expression of ATGL gene in
cells treated with the cultured supernatant of
Bifidobacterium breve BCRC 911145 (cells are cultured for
48 hours, the final concentration of the cultured supernatant
of *Bifidobacterium breve* BCRC 911145 in the medium for
15 culturing the cells is 0.125%). Compared to the Mock group,
*: $p < 0.01$.

FIG. 6B shows the relative expression of PLIN1 gene in
cells treated with the cultured supernatant of
Bifidobacterium breve BCRC 911145 (cells are cultured for
20 6 hours, the final concentration of the cultured supernatant
of *Bifidobacterium breve* BCRC 911145 in the medium for
culturing the cells is 0.25%). Compared to the Mock group,
***: $p < 0.001$.

FIG. 6C shows the relative expression of PPARG2 gene in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 24 hours, the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.125%). Compared to the Mock group, ***: $p < 0.001$.

FIG. 6D shows the relative expression of LIPE (HSL) gene in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 48 hours, the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.125%). Compared to the Mock group, *: $p < 0.05$.

FIG. 7A shows the relative expression of ATGL gene in cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 24 hours, the final concentration of the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.25%). Compared to the Mock group, *: $p < 0.05$.

FIG. 7B shows the relative expression of PLIN1 gene in cells treated with the dead bacteria suspension of

Bifidobacterium breve BCRC 911145 (cells are cultured for 24 hours, the final concentration of the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.25%). Compared to the Mock group, ***: $p < 0.001$.

FIG. 7C shows the relative expression of PPARG2 gene in cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 24 hours, the final concentration of the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.25%). Compared to the Mock group, ***: $p < 0.001$.

FIG. 7D shows the relative expression of CEBPA gene in cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 24 hours, the final concentration of the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.125%). Compared to the Mock group, ***: $p < 0.001$.

FIG. 8A shows the relative expression of CETP gene in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 6 hours, the final concentration of the cultured supernatant

of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.5%). Compared to the Mock group, ***: $p < 0.001$.

FIG. 8B shows the relative expression of SCARB1 gene
5 in cells treated with the cultured supernatant of
Bifidobacterium breve BCRC 911145 (cells are cultured for
6 hours, the final concentration of the cultured supernatant
of *Bifidobacterium breve* BCRC 911145 in the medium for
culturing the cells is 0.5%). Compared to the Mock group,
10 *: $p < 0.05$.

FIG. 8C shows the relative expression of ABCA1 gene
in cells treated with the cultured supernatant of
Bifidobacterium breve BCRC 911145 (cells are cultured for
6 hours, the final concentration of the cultured supernatant
15 of *Bifidobacterium breve* BCRC 911145 in the medium for
culturing the cells is 0.5%). Compared to the Mock group,
***: $p < 0.001$.

FIG. 8D shows the relative expression of LDLR gene in
cells treated with the cultured supernatant of
20 *Bifidobacterium breve* BCRC 911145 (cells are cultured for
48 hours, the final concentration of the cultured supernatant
of *Bifidobacterium breve* BCRC 911145 in the medium for
culturing the cells is 0.5%). Compared to the Mock group,

******: $p < 0.01$.

FIG. 9 show the relative expressions of CETP gene, SCARB1 gene and ABCA1 gene in cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 6 hours, the final concentration of the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.125%). Compared to the Mock group, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

FIG. 10 show the expression of glucose transporter type 4 (GLUT4) in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145. Compared to the Mock group, **: $p < 0.01$.

FIG. 11 show the relative expression of glucose transporter type 4 (GLUT4) in cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145. Compared to the Mock group, ***: $p < 0.001$.

FIG. 12 shows the results of the survey on the appetite status of the subjects in the experiment for efficacy test of viable bacteria powder. Compared to Week 0, *: $p < 0.05$, **: $p < 0.01$.

FIG. 13 shows the changes in total cholesterol of the subjects in the experiment for efficacy test of viable

bacteria powder. Compared to Week 0, *: $p < 0.05$. Total cholesterol reference value: <200 mg/dL.

FIG. 14A shows the changes in high-density lipoprotein cholesterol (HDL-C) of the subjects in the experiment for efficacy test of viable bacteria powder. Compared to Week 0, **: $p < 0.01$. High-density lipoprotein cholesterol (HDL-C) reference value: >40 mg/dL.

FIG. 14B shows the changes in low-density lipoprotein cholesterol (LDL-C) of the subjects in the experiment for efficacy test of viable bacteria powder. Low-density lipoprotein cholesterol (LDL-C) reference value: <100 mg/dL.

FIG. 14C shows the changes in the ratio of low-density lipoprotein cholesterol to high-density lipoprotein cholesterol (LDL/HDL) of the subjects in the experiment for efficacy test of viable bacteria powder. Reference values for the ratio of low-density lipoprotein cholesterol to high-density lipoprotein cholesterol: Male: <3.55 ; Female: <3.22 .

FIG. 15 shows the changes in fasting blood glucose of the subjects in the experiment for efficacy test of viable bacteria powder. Compared to Week 0, *: $p < 0.05$. Fasting blood glucose reference value: 74-109 mg/dL.

FIG. 16A shows the changes in body weight of subjects in the experiment for efficacy test of composition containing viable bacteria and soybean extract.

FIG. 16B shows the changes in body fat percentage of subjects in the experiment for efficacy test of composition containing viable bacteria and soybean extract.

FIG. 17 shows the changes in serum glucagon-like peptide-1 (GLP-1) concentrations of subjects in the experiment for blood glucose modulating efficacy test of composition containing viable bacteria, soybean extract and apple extract (Mode 1). For the same group of subjects, blood samples are collected at the same time intervals when the subjects do not take the composition of the present disclosure (the control group) and when the subjects take the composition of the present disclosure (the group taking the composition of the present disclosure). Compared to the baseline (fasting blood test value before the subject takes the composition of the present disclosure (not taking the composition of the present disclosure) (0 hour)), *: $p < 0.05$.

FIG. 18 shows the changes in blood glucose concentrations of subjects in the experiment for blood glucose modulating efficacy test of composition containing

viable bacteria, soybean extract and apple extract (Mode 1). For the same group of subjects, blood samples are collected at the same time intervals when the subjects do not take the composition of the present disclosure (the control group) and when the subjects take the composition of the present disclosure (the group taking the composition of the present disclosure). Compared to the baseline (fasting blood test value before the subject takes the composition of the present disclosure (not taking the composition of the present disclosure) (0 hour)), *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

FIG. 19 shows the changes in serum glucagon-like peptide-1 (GLP-1) concentrations of subjects in the experiment for blood glucose modulating efficacy test of composition containing viable bacteria, soybean extract and apple extract (Mode 2). For the same group of subjects, blood samples are collected at the same time intervals when the subjects do not take the composition of the present disclosure (the control group) and when the subjects take the composition of the present disclosure (the group taking the composition of the present disclosure).

FIG. 20A shows the changes in blood glucose concentrations of subjects in the experiment for blood

glucose modulating efficacy test of composition containing viable bacteria, soybean extract and apple extract (Mode 2). For the same group of subjects, blood samples are collected at the same time intervals when the subjects do not take the composition of the present disclosure (the control group) and when the subjects take the composition of the present disclosure (the group taking the composition of the present disclosure). Compared to the baseline (fasting blood test value before the subject takes the composition of the present disclosure (not taking the composition of the present disclosure) (0 hour)), ***: $p < 0.001$. Compared to the control group, #: $p < 0.05$.

FIG. 20B shows the changes in blood insulin concentrations of subjects in the experiment for blood glucose modulating efficacy test of composition containing viable bacteria, soybean extract and apple extract (Mode 2). For the same group of subjects, blood samples are collected at the same time intervals when the subjects do not take the composition of the present disclosure (the control group) and when the subjects take the composition of the present disclosure (the group taking the composition of the present disclosure). Compared to the baseline (fasting blood test value before the subject takes the composition of the

present disclosure (not taking the composition of the present disclosure) (0 hour)), **: $p < 0.01$.

FIG. 21 shows in the experiment for blood glucose modulating efficacy test of composition containing viable
5 bacteria, soybean extract and apple extract (Mode 2), changes in fasting blood glucose concentrations of subjects on the Day 5 and Day 8 (after taking the above composition for 3 consecutive days (Day 5 to Day 7)) of the experiment.

FIG. 22A shows survey results on the subjects' feeling
10 of hunger in the experiment for blood glucose modulating efficacy test of composition containing viable bacteria, soybean extract and apple extract (Mode 2).

FIG. 22B shows survey results on the subjects' appetite level in the experiment for blood glucose modulating
15 efficacy test of composition containing viable bacteria, soybean extract and apple extract (Mode 2).

FIG. 22C shows survey results on subjects' overall appetite changes in the experiment for blood glucose modulating efficacy test of composition containing viable
20 bacteria, soybean extract and apple extract (Mode 2).

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure may provide a probiotic, which

may be an isolated probiotic. The foregoing isolated probiotic of the present disclosure may be *Bifidobacterium breve*. In one embodiment, the foregoing isolated probiotic of the present disclosure may be a novel *Bifidobacterium*
5 *breve* which was deposited in the Bioresource Collection and Research Center (BCRC) of Food Industry Research and Development Institute (FIRDI), Taiwan, China on August 8, 2022, and obtained the deposit number BCRC 911145, and which was also deposited in the German Collection of
10 Microorganisms and Cell Cultures GmbH (DSMZ) on September 7, 2022 and obtained the deposit number DSM 34383 while for the sake of conciseness, this novel *Bifidobacterium breve* hereinafter will be referred to *Bifidobacterium breve* BCRC 911145 in the content of the
15 present disclosure.

Effects of the *Bifidobacterium breve* BCRC 911145 of the present disclosure may comprise, but is not limited to, reducing appetite, promoting glucagon-like peptide-1 (GLP-1) secretion, inhibiting adipocyte maturation, reducing fat
20 accumulation, promoting fat metabolism, reducing body weight, modulating blood lipids (such as reducing total cholesterol, promoting high-density lipoprotein cholesterol (HDL-C) synthesis, promoting low-density lipoprotein

cholesterol (LDL-C) metabolism), modulating blood glucose, etc., or any combination of the above.

There is no particular limitation on the application of the *Bifidobacterium breve* BCRC 911145 of the present disclosure, for example, the *Bifidobacterium breve* BCRC 911145 of the present disclosure may be applied to the fields related to health management and/or medical treatment etc., especially may be applied to the areas of appetite control, body weight control, body fat modulation, blood lipid modulation, blood glucose modulation, etc., but it is also not limited thereto.

In one embodiment, the aforementioned *Bifidobacterium breve* BCRC 911145 of the present disclosure may be prepared as an oral dosage form. Examples of the above-mentioned oral dosage form may include, but are not limited to, a powder, a granule, a tablet, a troche, a pill, a capsule, a lozenge, a solution, a suspension, an emulsion, a syrup, an elixir or a slurry, etc.

In one specific embodiment, the aforementioned *Bifidobacterium breve* BCRC 911145 of the present disclosure may be prepared as a powder for oral dosage form. In another specific embodiment, the aforementioned *Bifidobacterium breve* BCRC 911145 of the present

disclosure may be prepared as a capsule for oral dosage form. In yet another specific embodiment, the aforementioned *Bifidobacterium breve* BCRC 911145 of the present disclosure may be prepared as a solution for oral
5 dosage form.

The present disclosure may also provide a probiotic culture which is a culture of any foregoing probiotic of the present disclosure while the probiotic described herein may include *Bifidobacterium breve*. In one embodiment, the
10 probiotic described herein may be foregoing *Bifidobacterium breve* BCRC 911145.

The probiotic culture of the present disclosure mentioned above may comprise the aforementioned probiotic which has been cultured (i.e., the probiotic which
15 has been cultured alone), a cultured medium containing the aforementioned probiotic which has been cultured (i.e., the probiotic which has been cultured plus the cultured medium), or a cultured supernatant in which the aforementioned probiotic which has been cultured has been
20 removed (i.e., the cultured medium alone), but it is not limited thereto.

The manner of separating the foregoing probiotic which has been cultured from the cultured medium to obtain the

individual foregoing probiotic which has been cultured and foregoing cultured supernatant (cultured medium alone) is not particularly limited, as long as the two can be separated. For example, the foregoing probiotic which has been
5 cultured may be separated from the cultured medium by means such as centrifugation, filtration, or the like, but it is not limited thereto.

Moreover, short-chain fatty acid (SCFA) contained in the cultured medium or cultured supernatant in the probiotic
10 culture of the present disclosure may include at least one of the following, but is not limited to: formic acid, acetic acid, propionic acid, butyric acid, isobutyric acid, 3-methylbutyric acid, etc.

For the probiotic culture of the present disclosure
15 mentioned above, in one embodiment, the aforementioned probiotic which has been cultured may be active or maintain its activity, or may be a viable bacterium.

Furthermore, for the probiotic culture of the present disclosure mentioned above, in another embodiment, the
20 aforementioned probiotic which has been cultured may have been stripped of its own physiological activity (the bacterium loses its physiological functions), or may be a dead bacterium. There is no particular limitation on a

manner of making the aforementioned probiotic which has been cultured be stripped of its own physiological activity or became a dead bacterium, as long as it can make the aforementioned probiotic which has been cultured be
5 stripped of its physiological activity or became a dead bacterium. In one embodiment, the probiotic which has been cultured mentioned above can be made be stripped of its physiological activity or became a dead bacterium by a physical mean. For example, the probiotic which has been
10 cultured mentioned above can be made be stripped of its physiological activity (the bacterium loses its physiological functions) or became a dead bacterium by high temperature and/or high pressure, but it is not limited thereto.

15 In one embodiment, the probiotic culture of the present disclosure mentioned above may be the foregoing probiotic which has been cultured (i.e., the probiotic which has been cultured alone). For this embodiment, in one specific embodiment, the foregoing probiotic which has been
20 cultured may be active or maintain its activity, or may be a viable bacterium, however, in another specific embodiment, the foregoing probiotic which has been cultured may have been stripped of its own physiological activity (the

bacterium loses its physiological functions), or may be a dead bacterium.

In another embodiment, the probiotic culture of the present disclosure mentioned above may be a cultured medium containing the aforementioned probiotic which has been cultured (i.e., the probiotic which has been cultured plus the cultured medium). For this embodiment, in one specific embodiment, the foregoing probiotic which has been cultured may be active or maintain its activity, or may be a viable bacterium, however, in another specific embodiment, the foregoing probiotic which has been cultured may have been stripped of its own physiological activity (the bacterium loses its physiological functions), or may be a dead bacterium.

In further another embodiment, the probiotic culture of the present disclosure mentioned above may be a cultured supernatant in which the aforementioned probiotic have been removed (i.e., the cultured medium alone). For this embodiment, in one specific embodiment, the foregoing cultured supernatant may be sterilized and/or disinfected, however, in another specific embodiment, the foregoing cultured supernatant may be non-sterilized and/or non-disinfected. There is no particular limitation on a manner

for sterilizing and/or disinfecting the cultured supernatant, as long as it can make the cultured supernatant be sterilized and/or stripped of toxins that may be contained therein. For example, the cultured supernatant can be made be sterilized
5 and/or stripped of toxins that may be contained therein by high temperature and/or high pressure, but it is not limited thereto.

Furthermore, for the probiotic culture of the present disclosure mentioned above, there is also no particular
10 limitation on a medium used to culture the aforementioned probiotic to produce the probiotic culture of the present disclosure mentioned above, as long as it can make the aforementioned probiotic grow. Examples of a medium that can be used to culture the aforementioned probiotic to
15 produce the probiotic culture of the present disclosure mentioned above may include, but is not limited to, MRS broth (DeMan-Rogosa-Sharpe broth), Lysogeny broth (LB), skim milk medium, etc. For the probiotic culture of the present disclosure mentioned above, in one embodiment, a
20 medium used to culture the foregoing probiotic to produce the probiotic culture of the present disclosure mentioned above may be MRS broth.

In one embodiment, the probiotic culture of the present

disclosure mentioned above may be prepared as an oral dosage form. Examples of the above-mentioned oral dosage form may include, but are not limited to, a powder, a granule, a tablet, a troche, a pill, a capsule, a lozenge, a solution, a suspension, an emulsion, a syrup, an elixir or a slurry, etc.

In one specific embodiment, the aforementioned probiotic culture of the present disclosure may be prepared as a powder for oral dosage form. In another specific embodiment, the aforementioned probiotic culture of the present disclosure may be prepared as a capsule for oral dosage form. In yet another specific embodiment, the aforementioned probiotic culture of the present disclosure may be prepared as a solution for oral dosage form.

In addition, for any of the probiotic culture of the present disclosure mentioned above, in one specific embodiment, said probiotic may be the aforementioned *Bifidobacterium breve* BCRC 911145.

The probiotic culture of the present disclosure mentioned above may have effects of reducing appetite, promoting glucagon-like peptide-1 (GLP-1) secretion, inhibiting adipocyte maturation, reducing fat accumulation, promoting fat metabolism, reducing body weight,

modulating blood lipids (such as reducing total cholesterol, promoting high-density lipoprotein cholesterol (HDL-C) synthesis, promoting low-density lipoprotein cholesterol (LDL-C) metabolism), modulating blood glucose, etc., or
5 any combination of the above, but it is not limited thereto.

Moreover, there is also no particular limitation on the application of the probiotic culture of the present disclosure mentioned above. For example, the probiotic culture of the present disclosure mentioned above may be
10 applied to the fields related to health management and/or medical treatment etc., especially may be applied to the areas of appetite control, body weight control, body fat modulation, blood lipid modulation, blood glucose modulation, etc., but it is not limited thereto.

15 Based on the foregoing, the present disclosure may also provide the following uses of any of the aforementioned probiotic of the present disclosure or any of the aforementioned culture thereof, but it is not limited thereto:

20 (i) A use of any of the aforementioned probiotic of the present disclosure or any of the aforementioned culture thereof in the manufacture of a composition for reducing appetite and/or promoting glucagon-like peptide-1 (GLP-1)

secretion;

(ii) A use of any of the aforementioned probiotic of the present disclosure or any of the aforementioned culture thereof in the manufacture of a composition for inhibiting
5 adipocyte maturation;

(iii) A use of any of the aforementioned probiotic of the present disclosure or any of the aforementioned culture thereof in the manufacture of a composition for reducing fat accumulation and/or promoting fat metabolism;

10 (iv) A use of the aforementioned probiotic of the present disclosure or any of the aforementioned culture thereof in the manufacture of a composition for reducing body weight;

(v) A use of the aforementioned probiotic of the present
15 disclosure or any of the aforementioned culture thereof in the manufacture of a composition for modulating blood lipids. Said modulating blood lipids may comprise reducing total cholesterol, promoting high-density lipoprotein cholesterol (HDL-C) synthesis, promoting low-density
20 lipoprotein cholesterol (LDL-C) metabolism, etc., but it is not limited thereto; and/or

(vi) A use of the aforementioned probiotic of the present disclosure or any of the aforementioned culture

thereof in the manufacture of a composition for modulating blood glucose.

Furthermore, based on the foregoing, the present disclosure may further provide the following method, but it is not limited thereto:

(i) A method for reducing appetite and/or promoting glucagon-like peptide-1 (GLP-1) secretion, comprising, but not limited to, administering any of the probiotic of the present disclosure mentioned above or any of the culture thereof mentioned above to a subject in need thereof;

(ii) A method for inhibiting adipocyte maturation, comprising, but not limited to, administering any of the probiotic of the present disclosure mentioned above or any of the culture thereof mentioned above to a subject in need thereof;

(iii) A method for reducing fat accumulation and/or promoting fat metabolism, comprising, but not limited to, administering any of the probiotic of the present disclosure mentioned above or any of the culture thereof mentioned above to a subject in need thereof;

(iv) A method for reducing body weight, comprising, but not limited to, administering any of the probiotic of the present disclosure mentioned above or any of the culture

thereof mentioned above to a subject in need thereof;

(v) A method for modulating blood lipids, comprising, but not limited to, administering any of the probiotic of the present disclosure mentioned above or any of the culture thereof mentioned above to a subject in need thereof. The modulating blood lipids may comprise reducing total cholesterol, promoting high-density lipoprotein cholesterol (HDL-C) synthesis, promoting low-density lipoprotein cholesterol (LDL-C) metabolism, etc., but it is not limited thereto; and/or

(vi) A method for modulating blood glucose, comprising, but not limited to, administering any of the probiotic of the present disclosure mentioned above or any of the culture thereof mentioned above to a subject in need thereof.

The subject mentioned above may comprise a vertebrate, but it is not limited thereto. Moreover, the vertebrate mentioned above may include a fish, an amphibian, a reptile, a bird, or a mammal, but it is not limited thereto. Examples of the mammal may include, but are not limited to, a human, an orangutan, a monkey, a horse, a donkey, a llama, a goat, a pig, a dog, a cat, a rabbit, a guinea pig, a rat, and a mouse. In one embodiment, the subject mentioned

above may be a human.

Moreover, the present disclosure may further provide a probiotic composition. The foregoing probiotic composition of the present disclosure may comprise, but is not limited to, any of the probiotic of the present disclosure mentioned above or any of the culture thereof mentioned above and a prebiotic.

In the probiotic composition of the present disclosure, the weight ratio of the foregoing probiotic of the present disclosure or the culture thereof to the foregoing prebiotic may be about 1-6:1-12, such as about 1-5.5:1.5-11, about 1-5:1.5-10, about 1-4:2-9, about 1-3:3-5, about 1:1, about 1:1.5, about 1:2, about 1:2.5, about 1:3, about 1.5:1, about 2:1, about 2:1.5, about 2:3, about 3:1, about 3:8, about 4:7, about 5:6, about 6:5, about 6:7, but it is not limited thereto. In one embodiment, in the probiotic composition of the present disclosure, the weight ratio of the foregoing probiotic of the present disclosure or the culture thereof to the foregoing prebiotic may be about 1:1. In another embodiment, in the probiotic composition of the present disclosure, the weight ratio of the foregoing probiotic of the present disclosure or the culture thereof to the foregoing prebiotic may be about 1:1.5. In yet another

embodiment, in the probiotic composition of the present disclosure, the weight ratio of the foregoing probiotic of the present disclosure or the culture thereof to the foregoing prebiotic may be about 1:2.

5 The foregoing prebiotic may comprise at least one of the following ingredients, but it is not limited thereto: a soybean extract, an oat extract, a barley extract, an apple extract, a banana extract, a tomato extract, a chicory extract, an asparagus extract, a beet extract, a garlic
10 extract, an onion extract, a seaweed extract, a pectin extract, a tea extract, etc.

 In one embodiment, in the probiotic composition of the present disclosure, the foregoing prebiotic may be a soybean extract. In this embodiment, the weight ratio of the
15 foregoing probiotic of the present disclosure or the culture thereof to the foregoing soybean extract may be about 1-6:1-6, such as about 1-5:1-5, about 1.5-4:1.5-4, about 2-5:2-5, about 2.5-6:2.5-6, about 1:1, about 1:1.5, about 1:2, about 1:2.5, about 1:3, about 1:4, about 1.5:1, about 2:1,
20 about 2.5:1, about 3:1, about 4:1, about 2:3, about 3:2, about 5:6, about 6:5, but it is not limited thereto. Moreover, in one specific embodiment of this embodiment, the weight ratio of the foregoing probiotic of the present disclosure or

the culture thereof to the foregoing soybean extract may be about 1:1. In another specific embodiment of this embodiment, the weight ratio of the foregoing probiotic of the present disclosure or the culture thereof to the
5 foregoing soybean extract may be about 1:1.5.

In another embodiment, in the probiotic composition of the present disclosure, the foregoing prebiotic may comprise a soybean extract and an apple extract. In this embodiment, the weight ratio of the foregoing probiotic of
10 the present disclosure or the culture thereof, the foregoing soybean extract and the foregoing apple extract may be about 1-6:1-6:1-6, such as about 1-5:1-5:1-5, about 1.5-4:1.5-4:1.5-4, about 2-5:2-5:2-5, about 2.5-6:2.5-6:2.5-6, about 1:1:1, about 1:1.5:1.5, about 1:1.5:1, about 1:1:1.5,
15 about 1.5:1:1, about 1:2:2, about 1:2:1, about 1:1:2, about 2:2:1, about 2:1:1, about 1:2.5:2.5, about 1:2.5:1, about 1:1:2.5, about 2.5:1:1, about 1:3:3, about 1:3:1, about 1:1:3, about 3:1:1, about 1:4:4, about 1:4:1, about 1:1:4, about 4:1:1, about 2:3:3, about 3:2:2, about 5:6:6, about
20 6:5:5, but it is not limited thereto. Moreover, in one specific embodiment of this embodiment, the weight ratio of the foregoing probiotic of the present disclosure or the culture thereof, the foregoing soybean extract and the

foregoing apple extract may be about 1:1:1. In another specific embodiment of this embodiment, the weight ratio of the foregoing probiotic of the present disclosure or the culture thereto, the foregoing soybean extract and the foregoing apple extract may be about 1:1.5:1.5.

In addition, the probiotic composition of the present disclosure may be a food composition, a pharmaceutical composition, etc., but it is not limited thereto.

In one embodiment, the probiotic composition of the present disclosure may be a food composition. In this embodiment, the probiotic composition of the present disclosure may be prepared as an oral dosage form, but it is also not limited thereto.

In another embodiment, the probiotic composition of the present disclosure may be a pharmaceutical composition. In this embodiment, the probiotic composition of the present disclosure may also be prepared as an oral dosage form, but it is also not limited thereto.

The oral dosage form prepared by the probiotic composition of the present disclosure may include, but is not limited to, a powder, a granule, a tablet, a troche, a pill, a capsule, a lozenge, a solution, a suspension, an emulsion, a syrup, an elixir or a slurry, etc.

In one embodiment, the oral dosage form prepared by the probiotic composition of the present disclosure may be a powder. In another embodiment, the oral dosage form prepared by the probiotic composition of the present disclosure may be a capsule. In yet another embodiment, the oral dosage form prepared by the probiotic composition of the present disclosure may be a solution.

Moreover, in the embodiment in which the probiotic composition of the present disclosure may be a food composition, the probiotic composition of the present disclosure, in addition to the probiotic culture mentioned above and the prebiotic mentioned above, may further comprise an additive which is acceptable for food.

The foregoing additive which is acceptable for food may include, but is not limited to, a preservative, a bactericide, an antioxidant, a bleaching agent, a color retention agent, an expander, a coloring agent, a flavoring agent, a sweetener, a thickener (paste), a binder, an emulsifier, a carrier, etc., or any combination thereof.

Moreover, in the embodiment in which the probiotic composition of the present disclosure may be a pharmaceutical, the probiotic composition of the present disclosure, in addition to the probiotic culture mentioned

above and the prebiotic mentioned above, may further comprise a pharmaceutically acceptable carrier or salt, but it is not limited thereto.

The pharmaceutically acceptable carrier mentioned
5 above may comprise, but is not limited to, a solvent, a dispersion medium, a coating, an antibacterial and antifungal agent, or an isotonic and absorption delaying agent, etc. which is suitable for pharmaceutical administration. The pharmaceutical composition can be
10 formulated into dosage forms for different administration routes utilizing conventional methods.

Moreover, the pharmaceutically acceptable salt mentioned above may comprise, but is not limited to, salts including inorganic cation, such as alkali metal salts such
15 as sodium salt, potassium salt or amine salt, such as alkaline-earth metal salt such as magnesium salt or calcium salt, such as the salt containing bivalent or quadrivalent cation such as zinc salt, aluminum salt or zirconium salt. In addition, the pharmaceutically acceptable salt may also
20 be organic salt, such as dicyclohexylamine salt, methyl-D-glucamine, and amino acid salt such as arginine, lysine, histidine, glutamine, but it is not limited thereto.

EXAMPLES

A. Materials and Methods

1. Bacterial species identification

A strain isolated from the human intestine was subjected
5 to genome-wide average nucleotide identity analysis based
on the GenomesDB database using the software/tool for
average nucleotide identity (ANI) calculation, JSpeciesWS
online web server. The results are shown in Table 1 below.

Table 1

Type strains	Size	GC [%]	ANId value (%)
<i>Bifidobacterium breve</i> LMG 13208	2,263,780	58.88	97.93
<i>Bifidobacterium breve</i> BBRI4	2,426,720	58.72	97.74
<i>Bifidobacterium breve</i> BR3	2,426,006	59.09	97.81
<i>Bifidobacterium breve</i> GED8481	2,356,336	58.66	97.75
<i>Bifidobacterium breve</i> NCTC11815 [T]	2,275,664	58.89	97.95
<i>Bifidobacterium breve</i> 12L	2,244,624	58.87	98.03

<i>Bifidobacterium breve</i> 2L	2,240,722	58.85	98.03
<i>Bifidobacterium breve</i> 31L	2,265,654	58.57	98.06
<i>Bifidobacterium breve</i> 689b	2,331,707	58.73	97.98
<i>Bifidobacterium breve</i> ACS-071-V-Sch8b	2,327,492	58.73	98.43
<i>Bifidobacterium breve</i> CECT 7263	2,314,396	58.88	98.33
<i>Bifidobacterium breve</i> DSM 20213 = JCM 1192 DSM 20213	2,257,131	58.87	97.94
Isolated strain of the present disclosure	2,460,420	59.1	100

According to the results of the genome-wide average nucleotide identity analysis, it is known that the whole genome sequence of this isolated strain is 97.74% to 98.43% similar to that of *Bifidobacterium breve* subspecies (as shown in Table 1). Therefore, this isolated strain is confirmed to be a novel *Bifidobacterium breve*.

The novel *Bifidobacterium breve* was deposited in the Bioresource Collection and Research Center (BCRC) of

Food Industry Research and Development Institute (FIRDI), Taiwan, China on August 8, 2022, and the deposit number BCRC 911145 was obtained, and the novel *Bifidobacterium breve* was also deposited in the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ) on September 7, 2022 and the deposit number DSM 34383 was obtained. This novel *Bifidobacterium breve* hereinafter will be referred to *Bifidobacterium breve* BCRC 911145.

10 **2. Culturing of *Bifidobacterium breve* BCRC 911145 and acquisition of culture**

2-1. Culturing of *Bifidobacterium breve* BCRC 911145

2-1-1. Preparation of 5 L fermenter

3 L of MRS broth (DeMan-Rogosa-Sharpe broth) was prepared (55 g/L) in a 5 L fermenter using commercially available food-grade MRS broth powder (manufacturer: STBIO MEDIA, INC.). It was necessary to confirm that the powder in the prepared medium was completely dissolved.

The foregoing 5 L fermenter containing the medium was sterilized (121°C, 30 minutes). After the sterilization was completed, the fermenter was aerated (3 L/minute) and cooled to 37°C.

2-1-2. Fermentation in 5 L fermenter

After the preparation of fermenter mentioned above was completed, the fermenter was aerated (3 L/minute) and the medium was stirred (100 rpm) at 37°C for 20 minutes. At the same time, the dissolved oxygen electrode was
5 calibrated (100%).

Bifidobacterium breve BCRC 911145 could be implanted into the medium of the fermenter for performing fermentation only when the dissolved oxygen was less than 5%, and before implanting the bacterial strain, it was
10 necessary to confirm that the fermenter was not contaminated.

The conditions for bacterial strain implantation and fermentation are as follows:

(1) Absorbance at 600nm (OD_{600}) of bacterial strain
15 suspension for implantation in fermenter: 3.0-5.0;

(2) Amount of implanted bacteria: 5% (v/v);

(3) Dissolved oxygen: <1% (aerating with a small amount of nitrogen to maintain);

(4) Stirring speed: 25 rpm;

20 (5) Temperature: $37 \pm 1^\circ\text{C}$;

(6) pH: 6.0 ± 0.1 ;

(7) Culturing time: about 6-8 hours (culturing until the absorbance at 600 nm (OD_{600}) of the cultured medium in the

fermenter to be 3.0-4.0).

After fermentation was completed, samples were taken for inspection and photographed under a microscope to confirm the bacterial morphology and confirm bacterial strain activity.

After that, the fermenter was cooled (the temperature was set to 4°C, the actual temperature was less than 15°C).

2.2 Acquisition of culture

2-2-1. Acquisition of viable bacteria of cultured *Bifidobacterium breve* BCRC 911145

After the foregoing “2-1-2. Fermentation in 5 L fermenter” was completed, centrifugation was performed to remove the medium to obtain viable bacteria.

The centrifugation conditions are as follows:
10000 rpm, 10 minutes.

After centrifugation, the viable bacteria were lyophilized.

Afterwards, the lyophilized viable bacteria were pulverized to form viable bacteria powder.

2-2-2. Acquisition of cultured supernatant of cultured *Bifidobacterium breve* BCRC 911145

After the foregoing “2-1-2. Fermentation in 5 L

fermenter” was completed, the fermenter was sterilized (steam sterilization for 30 minutes).

After cooling, centrifugation was performed to remove the bacterial cells and obtain the cultured supernatant.

5 The centrifugation conditions are as follows:
10000 rpm, 10 minutes.

2-2-3. Acquisition of dead bacteria suspension of cultured *Bifidobacterium breve* BCRC 911145

After the foregoing “2-1-2. Fermentation in 5 L fermenter” was completed, the fermenter was sterilized (steam sterilization for 30 minutes).

After cooling, centrifugation was performed to remove the medium and obtain the dead bacteria.

15 The centrifugation conditions are as follows:
5000 rpm, 10 minutes.

After centrifugation, water in an amount equal to the volume of the culture before centrifugation was added to the dead bacteria to form a dead bacteria suspension.

20 3. Detection of glucagon-like peptide-1 (GLP-1) secreted by cells *in vitro*

3-1. Materials

(1) Cell line: NCI-H716 cells (ATCC, CCL-251);

(2) Medium:

RPMI 1640 Media (Gibco);

10% (v/v) Fetal bovine serum (Gibco);

2 mM L-Glutamine (Gibco);

5 1% (v/v) Penicillin-streptomycin (Gibco);

(3) Trypan blue dead cell stain (Lonza; Cat. 17-942E);

(4) PBS (Gibco);

(5) ELISA reader (BioTek);

(6) GLP-1 ELISA KIT (CEA804Mi).

10 **3-2. Methods**

(1) The cells were seeded in a 6-well cell culture plate (culture volume was 2 mL) at a density of 1×10^5 cells/well and cultured in a 37°C, 5% CO₂ incubator for 24 hours.

15 (2) Two days before the detection, 1×10^5 cells/well were seeded into a 24-well plate coated with Matrix gel, and the medium was replaced with DMEM complete medium and the cells were cultured for 24 hours;

(3) One day before the detection, the cell culture medium was changed to DMEM w/o glucose medium, and
20 forskolin (final concentration in the medium was 1 μM) and 3-isobutyl-1-methylxanthine (IBMX) (final concentration in the medium was 1 μM) were added at the same time and co-cultured for 4 hours;

(4) The supernatant in each well of the culture plate was removed, then PBS containing 1 mM CaCl_2 (300 μL /well) was added to each well, and different samples to be tested and 1 μM forskolin and 1 μM 3-isobutyl-1-methylxanthine were added. After that, culturing was performed for 24 hours;

(5) After 24 hours of culturing, the supernatant was aspirated from each well, and the GLP-1 content in the supernatant was detected using GLP-1 ELISA KIT (CEA804Mi) to confirm the amount of GLP-1 secreted by the cells into the medium;

(6) Statistical analysis was performed using Excel with one-tailed Student's t-test (T-TEST) (compared to Mock, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

15

4. Detection of PYY gene expression in cells *in vitro*

4-1. Materials and Instruments

(1) Cell line: NCI-H716 cells (ATCC, CCL-251);

(2) Medium:

20 89% (v/v) RPMI 1640 Media (Gibco);

10% (v/v) Fetal bovine serum (Gibco);

2 mM L-Glutamine (Gibco);

1% (v/v) Penicillin-streptomycin (Gibco);

(3) RNA Extraction Kit (Geneaid);

(4) SuperScript® III reverse transcriptase
(Invitrogen);

(5) Primer set: Primer set for PYY gene and primer set
5 for GAPDH gene as internal control;

(6) KAPA CYBR FAST qPCR Kits (2x) (KAPA
Biosystems);

(7) StepOnePlus Real-Time PCR System (ABI).

4-2. Methods

10 4-2-1. Cell culture and pre-treatment

(1) The cells were seeded in a 6-well cell culture plate
(culture volume was 2 mL) at a density of 1×10^5 cells/well
and cultured in a 37°C, 5% CO₂ incubator for 24 hours.

(2) Afterwards, the cells were treated with different
15 samples to be tested for 24 hours and 48 hours;

(3) The cells were harvested for performing gene
expression analysis.

4-2-2. Gene expression analysis

(1) RNA was extracted from cells using RNA Extraction
20 Kit (Geneaid);

(2) The above-mentioned RNA (2000 ng) was reverse
transcribed into cDNA using SuperScript® III Reverse
Transcriptase (Invitrogen);

(3) The cDNA was subjected to real-time polymerase chain reaction using KAPA SYBR FAST qPCR Kits (2x) (KAPA Biosystems) by StepOnePlus Real-Time PCR System to measure the expression level of the target gene;

5 (4) The melting curve during real-time polymerase chain reaction was analyzed;

(5) Relative quantification of gene expression was determined using the $2^{-\Delta\Delta C_T}$ method. The threshold cycle (C_T value) of the GAPDH reference gene was used as the
10 internal control and the simulation group, and the relative fold change was calculated according to the formulas shown in Formula (I) to Formula (III) below:

$$\Delta C_T = C_{T_{Target\ gene/Reference\ gene}} - C_{T_{Internal\ control}}$$

Formula (I);

15

$$\Delta\Delta C_T = \Delta C_{T_{Target\ gene}} - \Delta C_{T_{Reference\ gene}}$$

Formula (II);

$$Fold\ change = 2^{-\Delta\Delta C_{T\ Mean}}$$

20

Formula (III)

(6) The standard deviation of the relative expression levels of gene was calculated by STDEV in Excel;

(7) Statistical analysis was performed using Excel with

one-tailed Student's t-test (T-TEST) (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

5. Detection of the expressions of fat-related genes (CEBPA, UCP, ATGL, PLIN1, PPARG2 and LIPE(HSL)) in cells *in vitro*

5.1 Materials and Instruments

(1) Cell line: Mouse stroma bone marrow, stroma OP9
(BCRC; Cat. 60566);

10 (2) Medium:

Minimum essential medium α (MEM α) (Gibco;
Cat.12000-022, or an item with the same function);

2% (v/v) Fetal bovine serum (Gibco; Cat.
10437-028);

15 1% (v/v) Antibiotics (Gibco; Cat.15240-062,
or an item with the same function);

(3) 10X DPBS (Gibco; Cat. 14200-075, or an item with
the same function);

(4) Trypan blue dead cell stain (Lonza; Cat. 17-942E,
20 or an item with the same function);

(5) RB Buffer (Geneaid; Cat. RP050, or an item with
the same function);

(6) 10X Trypsin solution (SIGMA; Cat.59427C, or an

item with the same function);

(7) RNA Extraction Kit (Geneaid);

(8) SuperScript® III reverse transcriptase (Invitrogen);

5 (9) Primer set: Primer sets for each gene to be tested (CEBPA, UCP, ATGL, PLIN1, PPARG2 and LIPE(HSL)) and primer set for the GAPDH gene as an internal control;

(10) KAPA CYBR FAST qPCR Kits (2x) (KAPA Biosystems);

10 (11) StepOnePlus Real-Time PCR System (ABI).

5-2. Methods

5-2-1. Preparation of working reagents

(1) 1X DPBS: 10X DPBS was diluted 10-fold with sterilized ddH₂O;

15 (2) 1X Trypsin solution: 10X Trypsin solution was diluted 10-fold with 1X DPBS.

5-2-2. Cell culture and pre-treatment

(1) The cells were seeded in a 6-well cell culture plate at a density of 1×10^5 cells/well (culture volume was 2 mL) and cultured in a 37°C, 5% CO₂ incubator for 24 hours;

20 (2) After that, the cells were divided into Mock group and experimental groups (high-dose group and low-dose group), and after the addition of different samples to be

tested, the cells were placed in a 37°C, 5% CO₂ incubator for treatment for 6 hours, 24 hours and 48 hours;

(3) After the treatment was completed, the medium was removed;

5 (4) the cells were washed with 1X DPBS (1 mL/well) and then the 1X DPBS was removed;

(5) Step (4) was repeated;

(6) RB Buffer (600 µL/well) was added to lyse the cells to obtain a cell lysate solution;

10 (7) The cell lysate solution was collected in a 1.5 mL microcentrifuge tube and stored in a -80°C refrigerator.

5-2-3. Gene expression analysis

(1) RNA was extracted from cells using RNA Extraction Kit (Geneaid);

15 (2) The above-mentioned RNA (2000 ng) was reverse transcribed into cDNA using SuperScript® III Reverse Transcriptase (Invitrogen);

(3) The cDNA was subjected to real-time polymerase chain reaction using KAPA SYBR FAST qPCR Kits (2x) (KAPA Biosystems) by StepOnePlus Real-Time PCR System
20 to measure the expression level of the target gene;

(4) The melting curve during real-time polymerase chain reaction was analyzed;

(5) Relative quantification of gene expression was determined using the $2^{-\Delta\Delta C_T}$ method. The threshold cycle (C_T value) of the GAPDH reference gene was used as the internal control and the simulation group, and the relative
5 fold change was calculated according to the formulas shown in Formula (I) to Formula (III) above.

6. Detection of fat accumulation in cells *in vitro*

OP9 mouse embryonic stem cells were used as a cell
10 platform to confirm whether the test samples in the experimental group have the effect of reducing oil droplet formation.

6-1. Materials

(1) Cell line: OP9 mouse embryonic stem cell (ATCC;
15 Cat. CRL-2749);

(2) Medium:

79% (v/v) Minimum essential medium α
(MEM α) (Gibco; Cat. 12561072);

20 20% (v/v) Fetal bovine serum (Gibco; Cat. 10437-028);

1% (v/v) Penicillin-streptomycin (Gibco; Cat. 15140122);

(3) 10X DPBS (Gibco; Cat. 14200-075, or an item with

the same function);

(4) Trypan blue dead cell stain (Lonza; Cat. 17-942E, or an item with the same function);

(5) 10X Trypsin solution (Gibco; Cat. 15400-054);

5 (6) Oil red O (Sigma; Cat. O0625-25G);

(7) Isopropanol (ECHO CHEMICAL CO., LTD.; Cat. 184130010);

(8) Formaldehyde (ECHO CHEMICAL CO., LTD.; Cat. 119690010).

10 **6-2 Methods**

6-2-1. Preparation of working reagents

(1) 1X DPBS: 10X DPBS was diluted 10-fold with sterilized ddH₂O;

15 (2) 1X Trypsin solution: 10X Trypsin solution was diluted 10-fold with 1X DPBS;

(3) 10% Formaldehyde: Formaldehyde was diluted with sterilized ddH₂O;

(4) 50% and 60% Isopropanol: Isopropanol was diluted with sterilized ddH₂O;

20 (5) Oil red O stock solution: Oil Red O was prepared with 100% isopropanol to an Oil Red O stock solution with a final concentration of 3 mg/mL;

(6) Oil red O working solution: Oil Red O stock

solution was mixed with ddH₂O in a ratio of 3:2 to form a mixed solution. The mixed solution was then centrifuged at 1000 x g for 10 minutes and the supernatant was taken to a new 15 mL centrifuge tube for use.

5 **6-2-2. Cell culture and pre-treatment**

(1) The cells were seeded in a 24-well cell culture plate at a density of 8×10^4 cells/well (culture volume was 500 μ L/well) and cultured in a CO₂ incubator for 7 days, with replacing the medium every 3 days;

10 (2) After 7 days, the formation of oil droplets in the cells was observed under a microscope to confirm cell differentiation;

(3) The medium in the culture plate was replaced with the medium for the experimental groups (containing the test sample) and the medium for the control group (Mock), and
15 culturing was performed in a CO₂ incubator for 7-10 days, with replacing the medium every 3 days.

6-2-3. Determination of oil droplet content

(1) After the culture was completed, the liquid in the
20 well plate was removed and the cells were rinsed twice with 1X DPBS;

(2) 10% methanol (1 mL/well) was added to the cells and the cells was fixed for 30 minutes at room temperature;

(3) The liquid in the well plate was removed and the cells were rinsed twice with 1X DPBS;

(4) The liquid in the well plate was removed and 60% isopropanol (1 mL/well) was added and let stand at room
5 temperature for 1 minute;

(5) The liquid in the well plate was removed and Oil Red O working solution (1 mL/well) was added for staining for 1 hour at room temperature in the dark;

(6) The liquid in the well plate was removed and 60%
10 isopropanol (1 mL/well) was added for destaining for 5 seconds;

(7) The liquid in the well plate was removed and ddH₂O (1 mL/well) was added;

(8) Observation and photographing were performed
15 with a microscope;

(9) The liquid in the well plate was removed and 100% isopropanol was added, and the well plate was placed on an orbital shaker and shaken for 10 minutes to elute the dye;

(10) The liquid in the well plate was added to a 96-
20 well plate (100 µL/well) and the absorbance value at a wavelength of 510 nm (O.D. 510) thereof was determined by a spectrophotometer;

(11) Statistical analysis was performed using Excel

with one-tailed Student's t-test (T-TEST) (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

7. *In vitro* cellular lipolysis assay

5 OP9 mouse embryonic stem cells were used as a cell platform to confirm the effect of the stimulation of the test sample in the experimental group on the glycerol content released into the medium during the OP9 adipogenic differentiation, so as to know whether the test sample has
10 the effect of lipolysis.

7-1. Materials

(1) Cell line: OP9 mouse embryonic stem cell (ATCC; Cat. CRL-2749);

(2) Medium:

15 79% (v/v) Minimum essential medium α (MEM α) (Gibco; Cat. 12561072);

 20% (v/v) Fetal bovine serum (Gibco; Cat. 10437-028);

 1% (v/v) Penicillin-streptomycin (Gibco; Cat. 15140122);
20

(3) 10X DPBS (Gibco; Cat. 14200-075, or an item with the same function);

(4) Trypan blue dead cell stain (Lonza; Cat. 17-942E,

or an item with the same function);

(5) 10X Trypsin solution (Gibco; Cat. 15400-054);

(6) Glycerol cell-based assay kit (Cayman; Cat. 10011725):

5 Glycerol standard solution (Cayman; Cat. 10009952);

 Free glycerol assay reagent (Cayman; Cat. 10009953);

 Chloroquine positive control (25 mM) (Cayman; 10 Cat. 10012670).

7-2. Methods

7-2-1. Preparation of working reagents

(1) 1X DPBS: 1X DPBS: 10X DPBS was diluted 10-fold with sterilized ddH₂O;

15 (2) 1X Trypsin solution: 10X Trypsin solution was diluted 10-fold with 1X DPBS.

(3) Free glycerol assay reagent: The vial with this label (Cat. 10009953) in the kit was opened and 5 mL of ddH₂O was added to dissolve the content in the vial to prepare the 20 free glycerol assay reagent. The amount of one vial could supply 48 samples (half a 96-well plate). What prepared can be stored at 4°C for up to 2 weeks;

(4) Glycerol standard solution: The concentration of

the glycerol stock solution in the vial with this label (Cat. 10009952) in the kit was 125 µg/mL. This stock solution was serially diluted. Eight clean 1.5 mL microcentrifuge tubes were individually written with numbers 1 through 8. 5 200 µL of glycerol stock solution was added to tube 1, and 100 µL of 1X DPBS was added to each of the remaining 7 tubes. 100 µL was then taken from tube 1 to tube 2, mixed well, and then 100 µL was taken from the second tube to tube 3, and this operation was repeated to perform serially 10 dilution to tube 7 to respectively obtain glycerol solutions with the concentrations of 125 µg/mL (tube 1), 62.5 µg/mL (tube 2), 31.25 µg/mL (tube 3), 15.625 µg/mL (tube 4), 7.8125 µg/mL (tube 5), 3.90625 µg/mL (tube 6), 1.953125 µg/mL (tube 7), and 0 µg/mL (tube 8).

15 **7-2-2. Cell culture and pre-treatment**

(1) The cells were seeded in a 24-well cell culture plate at a density of 8×10^4 cells/well (culture volume was 500 µL/well) and cultured in a CO₂ incubator for 7 days, with replacing the medium every 3 days;

20 (2) After 7 days, the formation of oil droplets in the cells was observed under a microscope to confirm cell differentiation;

(3) The medium was replaced with the medium for the

experimental groups (the test samples) and the medium for the control group (Mock), and culturing was performed in a CO₂ incubator for 7-10 days, with replacing the medium every 3 days.

5 **7-2-3. Determination of glycerol content released into the medium**

(1) After the culture was completed, the cell cultured medium of the last culture was collected;

10 (2) 25 µL of the cell cultured medium, glycerol standard solution with various concentrations and chloroquine positive control were separately added to a 96-well plate;

(3) The free glycerol assay reagent (100 µL/well) was added and reacted at room temperature for 15 minutes;

15 (4) The absorbance value at a wavelength of 540 nm (O.D. 540) of each well was determined by a spectrophotometer;

(5) Statistical analysis was performed using Excel with one-tailed Student's t-test (T-TEST) (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

20

8. Detection of the expressions of blood lipids-related genes (CETP, SCARB1, ABCA1, LDLR) in cells *in vitro*

8.1 Materials and Instruments

(1) Cell line: Human hepatocellular carcinoma cell HepG2 (ATCC; Cat. HB-8065);

(2) Medium:

5 Dulbecco's modified Eagle's medium (DMEM) (Gibco; Cat. 11965-092);

10% (v/v) Fetal bovine serum (Gibco; Cat.10437-028, or an item with the same function);

1% (v/v) Penicillin-streptomycin (Gibco; Cat. 15140122);

(3) 10X DPBS (Gibco; Cat. 14200-075, or an item with the same function);

(4) Trypan blue dead cell stain (Lonza; Cat.17-942E, or an item with the same function);

15 (5) RB Buffer (Geneaid; Cat. RP050, or an item with the same function);

(6) 10X Trypsin solution (SIGMA; Cat.59427C, or an item with the same function);

(7) RNA Extraction Kit (Geneaid);

20 (8) SuperScript® III reverse transcriptase (Invitrogen);

(9) Primer set: Primer sets for each gene to be tested (CETP, SCARB1, ABCA1 and LDLR) and primer set for the

GAPDH gene as an internal control;

(10) KAPA CYBR FAST qPCR Kits (2x) (KAPA Biosystems);

(11) StepOnePlus Real-Time PCR System (ABI).

5 **8-2. Methods**

8-2-1. Cell culture and pre-treatment

(1) The cells were seeded in a 6-well plate at a density of 1×10^6 cells/well and cultured overnight (n=3);

(2) Cells were treated with different samples to be
10 tested for 6, 24, 48 hours;

(3) After removing the supernatant, the cells were washed once with 1X PBS;

(4) After the cells were lysed by adding 600 μ L of RB Buffer, the cell lysate solution was collected in a centrifuge
15 tube and stored in a -80°C refrigerator.

8-2-2. Gene expression analysis

(1) RNA was extracted from cells using RNA Extraction Kit (Geneaid);

(2) The above-mentioned RNA (2000 ng) was reverse
20 transcribed into cDNA using SuperScript® III Reverse Transcriptase (Invitrogen);

(3) The cDNA was subjected to real-time polymerase chain reaction using KAPA SYBR FAST qPCR Kits (2x)

(KAPA Biosystems) by StepOnePlus Real-Time PCR System to measure the expression level of the target gene;

(4) The melting curve during real-time polymerase chain reaction was analyzed;

5 (5) Relative quantification of gene expression was determined using the $2^{-\Delta\Delta C_T}$ method. The threshold cycle (C_T value) of the GAPDH reference gene was used as the internal control and the simulation group, and the relative fold change was calculated according to the formulas shown
10 in Formula (I) to Formula (III) above.

9. Detection of Glut4 expression level in cells *in vitro*

9.1 Materials and Instruments

(1) Cell line: Human hepatocellular carcinoma cell
15 HepG2 (ATCC; Cat. HB-8065);

(2) Medium:

Dulbecco's modified Eagle's medium (DMEM)
(Gibco; Cat. 12100046);

10% Fetal bovine serum (Gibco; Cat.10437-
20 028, or an item with the same function);

1% Antibiotics (Gibco; Cat.15240-062, or an
item with the same function);

Sodium bicarbonate (Sigma; Cat.S5761-500G,

or an item with the same function);

Sodium hydroxide (NaOH) (Sigma;
Cat.221465-500G, or an item with the same function);

Hydrogen chloride (HCl) (Sigma; Cat.7647-01-
5 0, or an item with the same function);

(3) 10X DPBS (Gibco; Cat. 14200-075, or an item with
the same function);

(4) Fetal bovine serum (Gibco; Cat.10437-028, or an
item with the same function);

10 (6) 10X Trypsin solution (Gibco; Cat.15400054, or an
item with the same function);

(7) Alexa Fluor 488 goat anti-mouse IgG antibody
(Invitrogen; Cat.A-11001, or an item with the same
function);

15 (8) GLUT4 antibody (Invitrogen; Cat.MA5-17176, or an
item with the same function);

(9) Insulin (Sigma; Cat.I9278-5ML, or an item with the
same function);

(10) Trypan blue dead cell stain (Lonza; Cat.17-942E,
20 or an item with the same function);

(11) Flow cytometer (BD, Accuri C6 Plus, or an item
with the same function).

9-2-1. Preparation of working reagents

(1) 1X DPBS: 10X DPBS was diluted 10-fold with sterilized ddH₂O;

(2) 1X Trypsin solution: 10X Trypsin solution was diluted 10-fold with pure water;

5 (3) 2% Fetal bovine serum solution: 1 mL Fetal bovine serum was mixed well with 49 mL 1X DPBS in a 50 mL centrifuge tube.

9-2-2. Cell culture and pre-treatment

(1) The cells were seeded in a 6-well cell culture plate
10 at a density of 1×10^5 cells/well (culture volume was 2 mL) and cultured in a 37°C, 5% CO₂ incubator for 24 hours;

(2) After that, the cells were divided into Mock group and experimental groups (high-dose group and low-dose group);

15 (3) Optional: The original medium was removed, and serum-free medium containing 1 μM insulin was added to the cells, and cultured in a 37°C, 5% CO₂ incubator for 72 hours. After that, the medium was removed;

(4) Medium containing different concentrations of test
20 samples was added to the cells and then the cells were cultured in a 37°C, 5% CO₂ incubator for 24 hours;

(5) Optional: 0.5 μM Insulin was added to the cells and the cells were cultured in a 37°C, 5% CO₂ incubator for 24

hours;

(6) After treatment was completed, the medium was removed;

(7) The cells were washed with 1X DPBS (1 mL/well)
5 and then the 1X DPBS was removed;

(8) Step (7) was repeated;

(9) 1X Trypsin solution (200 μ L/well) was added, the cells were placed in a 37°C, 5% CO₂ incubator for protecting it from light for 5 minutes, the culture plate was tapped and
10 detachment of the cells was confirmed;

(10) Medium (800 μ L/well) was added to terminate the action of trypsin;

(11) The cell suspension in each well was transferred to separate microcentrifuge tubes and centrifuged at 400 x
15 g for 5 minutes to remove the supernatant;

(12) 200 μ L 1X DPBS was added to each tube to wash the cells and then the tube was centrifuged at 400 x g for 5 minutes to remove the supernatant;

(13) 200 μ L of 2% Fetal bovine serum solution was
20 added to each tube and mixed well to fix the cells, and the cells were cultured at room temperature for 30-60 minutes in the dark;

(14) Each tube was centrifuged at 400 x g for 5 minutes

to remove the supernatant;

(15) 200 μ L 1X DPBS and 1 μ L GLUT4 antibody (1:200) were added to each tube and mixed well, and the cells are cultured at room temperature for 30 minutes in the dark;

5 (16) Each tube was centrifuged at 400 x g for 5 minutes to remove the supernatant;

(17) 200 μ L 1X DPBS was added to each tube to wash the cells and then the tube was centrifuged at 400 x g for 5 minutes to remove the supernatant;

10 (18) Step (17) was repeated;

(19) 200 μ L 1X DPBS and 1 μ L Alexa Fluor 488 goat anti-mouse IgG secondary antibody (1:200) were added to each tube and mixed well, and the cell were cultured at room temperature for 10 minutes in the dark;

15 (20) Each tube was centrifuged at 400 x g for 5 minutes to remove the supernatant;

(21) 200 μ L 1X DPBS was added to each tube to wash the cells and then the tube was centrifuged at 400 x g for 5 minutes to remove the supernatant;

20 (22) Step (21) was repeated;

(23) 200 μ L 1X DPBS was added to each tube to resuspend the cells;

(24) The fluorescence intensity of fluorescein

isothiocyanate (FITC) was analyzed by flow cytometry;

(25) Statistical analysis was performed using Excel with one-tailed Student's t-test (T-TEST) (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

5

10. Preparation of soybean extract

FUJI OIL CO., LTD, Japan was commissioned to produce the soybean extract.

The main processes are shown in sequence as follows:

- 10 (1) Soy protein isolation;
(2) Enzymatic hydrolysis;
(3) Isolation;
(4) Sterilization;
(5) Filtration;
15 (6) Spray drying; and
(7) Obtaining products.

11. Preparation of apple extract

20 The main processes of the apple extract are shown in sequence as follows:

- (1) Apples were coarsely chopped (coarsely chopped hole size: 12 mm);
(2) The coarsely chopped apples were extracted with

RO water to obtain a crude extract. The extraction conditions are as follows:

Weight ratio of coarsely chopped apples to RO water:
1:6;

5 Extraction temperature: $85 \pm 5^\circ\text{C}$;

Extraction time: 60 minutes;

Reflux pump $3.0 \pm 0.5 \text{ kg/cm}^2$;

(3) The crude extract was filtered (pore size: 400 mesh) and sampled for testing after filtration (Brix: 8.0 ± 0.5
10 (20°C));

(4) The filtrate was concentrated and sampled to confirm whether the concentrated liquid meets the predetermined specifications (Brix sugar: 8.0 ± 0.5 ; CA: 0.3 ± 0.2 (20°C); pH: 2.7 ± 1.0 (20°C));

15 (5) After reaching the specifications, the concentrated liquid could be used as the apple extract used in the present disclosure.

B. Ingredient analysis

20 1. Analysis of short-chain fatty acids (SCFA) contained in the cultured supernatant of cultured *Bifidobacterium breve* BCRC 911145

It is currently known that short-chain fatty acids

stimulate the secretion of glucagon-like peptide-1 (GLP-1). Therefore, the short-chain fatty acids contained in the cultured supernatant of cultured *Bifidobacterium breve* BCRC 911145 were analyzed to learn the potential of the

5 cultured supernatant of cultured *Bifidobacterium breve* BCRC 911145 to stimulate the secretion of glucagon-like peptide-1 (GLP-1).

The results are shown in Table 2.

Table 2: Short-chain fatty acids (SCFAs) contained in

10 the cultured supernatant of cultured *Bifidobacterium breve* BCRC 911145

Short-chain fatty acids	C1	C2	C3	C4	isoC4	isoC5
	Formic acid	Acetic acid	Propionic acid	Butyric acid	Isobutyric acid	3-Methylbutyric acid
(μ M)	11121.571	303688.219	790.959	178.32	88.731	10.291

According to Table 2, it is known that the cultured supernatant of cultured *Bifidobacterium breve* BCRC

15 911145 contains at least short-chain fatty acids such as

formic acid, acetic acid, propionic acid, butyric acid, isobutyric acid and 3-methylbutyric acid, and therefore should have the ability to stimulate the secretion of glucagon-like peptide-1.

5

C. In vitro experiments

1. Confirmation of the effects of cultured supernatant and dead bacteria suspension in promoting the secretion of glucagon-like peptide-1 (GLP-1) and reducing appetite

10 Glucagon-like peptide-1 (GLP-1) is the most important incretin in the human body, which can promote insulin secretion and inhibit glucagon secretion, and have the effect of regulating blood glucose, reducing appetite, and controlling body weight, while PYY gene is an appetite
15 suppression-related gene.

 The effects of *Bifidobacterium breve* BCRC 911145 in appetite control, weight control and blood glucose modulation were evaluated by analyzing the effects of cultured supernatant and dead bacteria suspension of
20 *Bifidobacterium breve* BCRC 911145 on glucagon-like peptide-1 (GLP-1) secretion and PYY gene expression.

 According to the methods described in “**3. Detection of glucagon-like peptide-1 (GLP-1) secreted by cells in**

vitro” and “4. Detection of PYY gene expression in cells *in vitro*” in “A. Materials and Methods” above, the effects of cultured supernatant and dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 (tested with NCI-H716 cells) on glucagon-like peptide-1 secretion and PYY gene expression were confirmed, respectively.

FIG. 1A and Table 3 show the relative amount of glucagon-like peptide-1 (GLP-1) secreted into the medium by the cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.25%).

FIG. 1B and Table 4 show the relative expression of PYY gene in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 48 hours, the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for culturing cells is 0.5%).

FIG. 2A and Table 5 show the relative amount of glucagon-like peptide-1 (GLP-1) secreted into the medium by the cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 (the final

concentration of the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.125%).

FIG. 2B and Table 6 show the relative expression of PYY gene in cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 24 hours, the final concentration of the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 in the medium for culturing cells is 0.125%).

Table 3: Relative amount of glucagon-like peptide-1 (GLP-1) secreted into the medium by the cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.25%)

		Relative content of GLP-1	Standard deviation	T-TEST
Mock	Mock	100	5.667436084	
Glucose (Positive control)	30 mM Glucose	138.5623	3.73147136	***
<i>Bifidobacterium breve</i> BCRC 911145	Cultured supernatant 0.25%	151.7905	2.109478447	***

Compared to the Mock group, ***: $p < 0.001$.

Table 4: Relative expression of PYY gene in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 48 hours, the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for culturing cells is 0.5%)

	Relative expression of PYY gene	Standard deviation
Mock	1	0
<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.5%	1.797643198	0.359497822

Table 5: Relative amount of glucagon-like peptide-1 (GLP-1) secreted into the medium by the cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 (the final concentration of the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 in the medium for culturing the cells is 0.125%)

		Relative content of GLP-1	Standard deviation	T-TEST
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Mock	Mock	100	2.897637825	
Glucose (Positive control)	30 mM Glucose	133.2586786	13.31189218	*
<i>Bifidobacterium</i> <i>breve</i> BCRC 911145	Dead bacteria suspension 0.125%	120.4927212	10.28495385	*

Compared to the Mock group, **: $p < 0.05$.

Table 6: Relative expression of PYY gene in cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 24 hours, the final concentration of the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 in the medium for culturing cells is 0.125%)

	Relative expression of PYY gene	Standard deviation
Mock	1	0
<i>Bifidobacterium breve</i> BCRC 911145 dead bacteria suspension 0.125%	1.484153968	0.094467242

Based on the results mentioned above, it is know that

both the cultured supernatant and the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 can effectively promote cells to secrete glucagon-like peptide-1 (GLP-1) and effectively enhance the expression of PYY gene in cells.

Specifically, the cultured supernatant of *Bifidobacterium breve* BCRC 911145 can promote the secretion of glucagon-like peptide-1 (GLP-1), with the secretion amount increased by 51.8%. At the same time, it can effectively increase the expression of the appetite suppression-related gene, PYY gene, with the expression increased by up to 79.8%.

Similarly, the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 can also promote the secretion of glucagon-like peptide-1 (GLP-1), with the secretion amount increased by 20.5%. At the same time, it can effectively increase the expression of the appetite suppression-related gene, PYY gene, with the expression increased by up to 48.4%.

In summary, both the cultured supernatant and the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 can be used for applications related to effects of appetite control, weight control and/or blood glucose modulation,

etc.

2. Confirmation of the effect of cultured supernatant in inhibiting adipocyte maturation and reducing fat accumulation

According to the methods described in “**5. Detection of the expression of fat-related genes (CEBPA, UCP, ATGL, PLIN1, PPARG2 and LIPE(HSL)) in cells *in vitro***” and “**6. Detection of fat accumulation in cells *in vitro***” in “A. Materials and Methods” above, the effects of cultured supernatant of *Bifidobacterium breve* BCRC 911145 on C/EBPA gene expression and fat accumulation were confirmed.

C/EBPA gene is a key regulatory gene for energy homeostasis and lipid storage in the body. Its low expression will terminate the differentiation of lipid droplet cells.

The abilities of *Bifidobacterium breve* BCRC 911145 in inhibiting adipocyte maturation and reducing fat accumulation were evaluated by analyzing the effects of cultured supernatant of *Bifidobacterium breve* BCRC 911145 on C/EBPA gene expression and fat accumulation.

The results are shown in Table 7 and Table 8 and FIG.

3A and FIG. 3B.

Table 7 and FIG. 3A show the relative expression of C/EBPA gene in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 6 hours, the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for culturing cells is 0.25%).

Table 8 and FIG. 3B show the Oil red (lipid) content in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145.

Table 7: Relative expression of C/EBPA gene in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 6 hours, the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for culturing cells is 0.25%)

	Relative expression level of C/EBPA gene	Standard deviation
Mock	1	0
<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.25%	0.165920988	0.007281205

Table 8: Oil red (lipid) content in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for culturing cells is 0.25%)

	Oil red (lipid) content in cells	Standard deviation
Mock	100	3.79
<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.25%	78.97	1.24

According to the results mentioned above, it is known that the cultured supernatant of *Bifidobacterium breve* BCRC 911145 is capable of effectively inhibiting the expression of C/EBPA gene, i.e., inhibiting adipocyte maturation, with an inhibition rate as high as 83.4%, and successfully reducing the fat accumulation by 21.0%.

In summary, the cultured supernatant of *Bifidobacterium breve* BCRC 911145 can be used for applications related to fat reduction, etc.

3. Confirmation of the effects of cultured supernatant

and dead bacteria suspension in promoting fat burning and/or lipolysis

According to the methods described in “**5. Detection of the expression of fat-related genes (CEBPA, UCP, ATGL, 5 PLIN1, PPARG2 and LIPE(HSL)) in cells *in vitro***” and “**7. *In vitro* cellular lipolysis assay**” in “**A. Materials and Methods**” above, the effects of cultured supernatant of *Bifidobacterium breve* BCRC 911145 on UCP1 gene and UCP2 gene expressions and lipolysis were confirmed.

10 Elevated expressions of UCP1 gene and UCP2 gene contribute to the conversion of adipocytes and promote fat burning.

The potential of *Bifidobacterium breve* BCRC 911145 in promoting fat burning and lipolysis was evaluated by 15 analyzing the effects of cultured supernatant and dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 on UCP1 gene and UCP2 gene expressions and the effect of cultured supernatant of *Bifidobacterium breve* BCRC 911145 on lipolysis.

20 The results are shown in Table 9, Table 10 and Table 11, and FIG. 4A, FIG. 4B and FIG. 5.

Table 9 and FIG. 4A show the relative expressions of UCP1 gene and UCP2 gene in cells treated with the cultured

supernatant of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 24 hours, the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for culturing cells is 0.125%).

5 Table 10 and FIG. 4B show the amount of glycerol in the cultured medium of cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for
10 culturing cells is 0.25%).

Table 11 and FIG. 5 show the relative expressions of UCP1 gene and UCP2 gene in cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 24 hours, the final concentration of
15 the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 in the medium for culturing cells is 0.25%).

Table 9: Relative expressions of UCP1 gene and UCP2 gene in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (cells are cultured for
20 24 hours, the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for culturing cells is 0.125%)

	Relative expression level
--	----------------------------------

Group/Gene	UCP1	UCP2
Mock	1	1
<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.125%	5.350352	2.839444
	Standard deviation	
Mock	0	0
<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.125%	1.793952	0.277548

Table 10: Amount of glycerol in the cultured medium of cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145 (the final concentration of the cultured supernatant of *Bifidobacterium breve* BCRC 911145 in the medium for culturing cells is 0.25%)

	Amount of glycerol in the cultured medium (%)	Standard deviation	T-TEST
Mock	100.00	8.05	
<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.25%	120.56	4.25	*

Compared to the Mock group, **: $p < 0.05$.

Table 11: Relative expressions of UCP1 gene and UCP2 gene in cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 (cells are cultured for 24 hours, the final concentration of the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 in the medium for culturing cells is 0.25%)

	Relative expression level	
Group/Gene	UCP1	UCP2
Mock	1	1
<i>Bifidobacterium breve</i> BCRC 911145 dead bacteria suspension 0.25%	2.332464	2.100877
	Standard deviation	
Mock	0	0
<i>Bifidobacterium breve</i> BCRC 911145 dead bacteria suspension 0.25%	0.099721	0.195996

Based on the results mentioned above, it is known that both the cultured supernatant and the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 can effectively promote fat burning and have the effect of reducing fat.

Specifically, the cultured supernatant of

Bifidobacterium breve BCRC 911145 can effectively increase the expressions of fat burning related genes, UCP1 gene and UCP2 gene, with the highest increase rate reaching about 4.35 times, and at the same time can increase the lipolysis efficiency by about 20.6%.

Similarly, the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 can also effectively increase the expressions of fat burning related genes, UCP1 gene and UCP2 gene, with the highest increase rate reaching about 1.3 times.

In summary, both the cultured supernatant and the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 can be used for applications related to fat reduction, etc.

4. Confirmation of the effects of cultured supernatant and dead bacteria suspension in metabolizing fat

According to the methods described in “5. Detection of the expression of fat-related genes (CEBPA, UCP, ATGL, PLIN1, PPARG2 and LIPE(HSL)) in cells *in vitro*” in “A. Materials and Methods” above, the effects of cultured supernatant of *Bifidobacterium breve* BCRC 911145 on fat-related gene expressions were confirmed.

ATGL is involved in the first step of fat metabolism in

lipid droplet cells, which can hydrolyze triglycerides. PLIN1 gene regulates fat metabolism in lipid droplet cells, and its low expression will promote fat metabolism in cells. PPARG2 regulates the β -oxidation process of fatty acids, and its low expression will promote fat metabolism. LIPE (HSL) hydrolyzes triglycerides into free fatty acids, which is responsible for converting cholesterol esters into free cholesterol. CEBPA terminates the differentiation of lipid droplet cells and is one of the key regulatory genes for energy homeostasis and lipid storage in the body.

The effect of *Bifidobacterium breve* BCRC 911145 on promoting fat metabolism was evaluated by analyzing the effects of the cultured supernatant and dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 on the expression of each of the above genes.

The results are shown in Table 12 and Table 13, and FIGS. 6A to 6D and FIGS. 7A to 7D.

Table 12 and FIGS. 6A to 6D show the relative expressions of ATGL gene, PLIN1 gene, PPARG2 gene and LIPE (HSL) gene in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145.

Table 13 and FIGS. 7A to 7D show the relative expressions of ATGL gene, PLIN1 gene, PPARG2 gene and

CEBPA gene in cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145.

Table 12: Relative expressions of ATGL gene, PLIN1 gene, PPARG2 gene and LIPE (HSL) gene in cells treated
5 with the cultured supernatant of *Bifidobacterium breve* BCRC 911145

Culture time	Gene Group	Relative expression level			
		ATGL	PLIN1	PPARG2	LIPE (HSL)
	Mock	1	1	1	1
6 hours	<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.25%		0.177391		
24 hours	<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.125%			0.187532	
48 hours	<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.125%	1.387792			4.383512
		Standard deviation			

	Mock	0	0	0	0
6 hours	<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.25%		0.019206		
24 hours	<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.125%			0.054401	
48 hours	<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.125%	0.230635			0.607965

Table 13: Relative expressions of ATGL gene, PLIN1 gene, PPARG2 gene and CEBPA gene in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC

5 911145

		Relative expression level			
Culture time	Group/Gene	ATGL	PLIN1	PPARG2	CEBPA
6 hours	Mock	1	1	1	1

24 hours	<i>Bifidobacterium breve</i> BCRC 911145 dead bacteria suspension 0.125%				0.642016
24 hours	<i>Bifidobacterium breve</i> BCRC 911145 dead bacteria suspension 0.25%	1.067559	0.770049	0.889962	
		Standard deviation			
6 hours	Mock	0	0	0	0
24 hours	<i>Bifidobacterium breve</i> BCRC 911145 dead bacteria suspension 0.125%				0.044377
24 hours	<i>Bifidobacterium breve</i> BCRC 911145 dead bacteria suspension 0.25%	0.042945	0.052777	0.024701	

Based on the results mentioned above, it is known that both the cultured supernatant and the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 can effectively promote fat metabolism and have the effect of

reducing fat.

Specifically, the cultured supernatant of *Bifidobacterium breve* BCRC 911145 can effectively promote fat metabolism, with the highest increase rate
5 reaching about 3.39 times.

Similarly, the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 can also effectively promote fat metabolism, with an average increase rate of about 10%.

10 In summary, both the cultured supernatant and the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 can be effectively used for applications related to fat reduction, etc.

15 **5. Confirmation of the effects of cultured supernatant and dead bacterial suspension in modulating blood lipids and promoting high-density lipoprotein cholesterol (HDL-C)**

According to the methods described in “**8. Detection of
20 the expression of blood lipids-related genes (CETP, SCARB1, ABCA1, LDLR) in cells *in vitro***” in “A. Materials and Methods” above, the effects of cultured supernatant and dead bacteria suspension of *Bifidobacterium breve* BCRC

911145 on blood lipids-related gene expressions were confirmed.

CETP gene, SCARB1 gene, and ABCA1 gene are related to the synthesis of high-density lipoprotein cholesterol (HDL-C), while the LDLR gene is related to the metabolism of low-density lipoprotein cholesterol (LDL-C).

The effects of *Bifidobacterium breve* BCRC 911145 in regulating blood lipids was evaluated by analyzing the effects of cultured supernatant and dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 on the expression of each of the above genes.

The results are shown in Table 14 and Table 15, and FIGS. 8A to 8D and FIG. 9.

Table 14 and FIGS. 8A to 8D show the relative expressions of CETP gene, SCARB1 gene, ABCA1 gene and LDLR gene in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145.

Table 15 and FIG. 9 show the relative expressions of CETP gene, SCARB1 gene and ABCA1 gene in cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145.

Table 14: Relative expressions of CETP gene, SCARB1 gene, ABCA1 gene and LDLR gene in cells treated with the

cultured supernatant of *Bifidobacterium breve* BCRC
911145

Culture time	Gene Group	Relative expression level			
		CETP	SCARB1	ABCA1	LDLR
6 hours	Mock	1	1	1	1
6 hours	<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.5%	1.724851	1.179249	4.069225	
48 hours	<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.5%				10.20103
		Standard deviation			
6 hours	Mock	0	0	0	0
6 hours	<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.5%	0.132365	0.12391	0.442441	
48 hours	<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.5%				2.247979

Table 15: Relative expressions of CETP gene, SCARB1 gene and ABCA1 gene in cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145

Culture time	Gene Group	Relative expression level		
		CETP	SCARB1	APA1
6 hours	Mock	1	1	1
6 hours	<i>Bifidobacterium breve</i> BCRC 911145 dead bacteria suspension 0.125%	1.896516	1.3157348	1.621850302
		Standard deviation		
6 hours	Mock	0	0	0
6 hours	<i>Bifidobacterium breve</i> BCRC 911145 dead bacteria suspension 0.125%	0.153532	0.1588998	0.054549114

5

Based on the results mentioned above, it is known that both the cultured supernatant and the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 can effectively modulate blood lipids and promote the synthesis of high-density lipoprotein cholesterol.

10

Specifically, the cultured supernatant of *Bifidobacterium breve* BCRC 911145 can effectively promote the synthesis of high-density lipoprotein cholesterol, with the increase rate reaching up to about 9.2 times, and can also promote the metabolism of low-density lipoprotein cholesterol.

Similarly, the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 can also effectively promote fat metabolism and the synthesis of high-density lipoprotein cholesterol, with the increase rate reaching up to about 89.7%.

6. Confirmation of the effects of cultured supernatant and dead bacteria suspension in modulating blood glucose

According to the methods described in “**9. Detection of Glut4 expression in cells *in vitro***” above, the effects of cultured supernatant and dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 on blood glucose-related gene expression were confirmed.

It is currently known that glucose transporter type 4 (GLUT4) is a member of the family of integral membrane glucose transporters (GLUT). It is expressed in muscle, fat and liver tissues, and has the function of balancing blood

glucose by the drawing effect on glucose to bring glucose into cells for use.

The effect of *Bifidobacterium breve* BCRC 911145 on modulating blood glucose was evaluated by analyzing the effects of cultured supernatant and dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 on the expression of glucose transporter type 4 (GLUT4).

The results are shown in Table 16 and Table 17, and FIG. 10 and FIG. 11.

Table 16 and FIG. 10 show the relative expression of glucose transporter type 4 (GLUT4) in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145.

Table 17 and FIG. 11 show the relative expression of glucose transporter type 4 (GLUT4) in cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145.

Table 16: Relative expression of glucose transporter type 4 (GLUT4) in cells treated with the cultured supernatant of *Bifidobacterium breve* BCRC 911145

	Relative expression of glucose transporter type 4 (GLUT4) (%)	Standard deviation	T-TEST
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Mock	100.00	5.671702896	
<i>Bifidobacterium breve</i> BCRC 911145 cultured supernatant 0.25%	120.40	3.591077438	**

Compared to the Mock group, **: $p < 0.01$.

Table 17: Relative expression of glucose transporter type 4 (GLUT4) in cells treated with the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145

	Relative expression of glucose transporter type 4 (GLUT4)	Standard deviation	T-TEST
Mock	1	0	
<i>Bifidobacterium breve</i> BCRC 911145 dead bacteria suspension 0.25%	2.156835291	0.629533696	***

Compared to the Mock group, ***: $p < 0.001$.

Based on the results mentioned above, both the cultured supernatant and the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 can effectively modulate blood glucose.

Specifically, the cultured supernatant of *Bifidobacterium breve* BCRC 911145 can increase the expression of glucose transporter type 4 (GLUT4) by about 20.4%.

Similarly, the dead bacteria suspension of *Bifidobacterium breve* BCRC 911145 can increase the expression of glucose transporter type 4 (GLUT4) by about 116%.

D. Human trials

D-1. Efficacy test of viable bacteria powder

Aims of the experiment: To test the weight loss effect of *Bifidobacterium breve* BCRC 911145

Experimental content:

1. Dosage form and dosage: *Bifidobacterium breve* BCRC 911145 viable bacteria capsule (100 mg viable bacteria powder/capsule, equivalent to 1×10^9 CFU/capsule), 1 capsule per day after dinner.

2. Experimental Design:

One viable bacteria capsule was taken daily for 4 consecutive weeks and the tests were performed before and 4 weeks after administration.

3. Conditions for subject:

Healthy adults over 20 years of age, known to have >25% body fat in males; >30% body fat in females.

4. Number of subjects: 9 persons.

5. Test Items:

5 (1) Survey-Appetite Status;

(2) Blood biochemical indexes (total cholesterol, HDL, LDL, LDL/HDL and fasting blood glucose) (Le Zen Reference Lab was entrusted for testing).

The results are shown in FIG. 12, FIG. 13, FIG. 14A and
10 FIG. 14B, and Table 18.

FIG. 12 shows the results of the survey on the appetite status of the subjects.

Based on FIG. 12, it is known that after continuously taking *Bifidobacterium breve* BCRC 911145 viable bacteria
15 capsules for 4 weeks, sporadic food cravings can be significantly reduced by 25.9% and hunger can be significantly reduced by 20.4%. In addition, the survey results also show that the proportion of respondents whose sporadic food cravings and hunger symptoms are improved
20 is 62.5% and 87.5% of the total number of people, respectively.

Table 18: Blood biochemical indexes of subjects

Glucose	Mean	Standard deviation	%	P value	Total number of respondents	9
Week 0	94.4	3.0			Number of respondents who improved	9
Week 4	85.1	2.5	-9.9	0.027182	Proportion of respondents who improved (%)	100
Cholesterol	Mean	Standard deviation	%	p value	Total number of respondents	9
Week 0	206.6	11.0			Number of respondents who improved	7
Week 4	198	8.8	-4.2	0.272127	Proportion of respondents who improved (%)	77.8
HDL	Mean	Standard deviation	%	p value	Total number of respondents	9
Week 0	53.85556	3.4			Number of respondents who improved	8

Week 4	55.97778	3.5	-3.9	0.0055	Proportion of respondents who improved	88.9
LDL	Mean	Standard deviation	%	p value	Total number of respondents	9
Week 0	138.9	9.7			Number of respondents who improved	7
Week 4	127.6	9.6	-8.2	0.15	Proportion of respondents who improved (%)	77.8
LDL/HDL	Mean	Standard deviation	%	p value	Total number of respondents	9
Week 0	2.6	0.3			Number of respondents who improved	7
Week 4	2.4	0.3	-9.3	0.101	Proportion of respondents who improved (%)	77.8

Based on FIG. 13 and Table 18, it is known that after continuously taking *Bifidobacterium breve* BCRC 911145 viable bacteria capsules for 4 weeks, total cholesterol can

be lowered by 4.2% and the proportion of respondents who improved reaches 77.8%.

Furthermore, according to FIGS. 14A to 14C and Table 18, it is known that after continuously taking
5 *Bifidobacterium breve* BCRC 911145 viable bacteria capsules for 4 weeks, high-density lipoprotein cholesterol (HDL-C) can be significantly increased by 3.9% and low-density lipoprotein cholesterol (LDL-C) can be reduced by 8.2%, while reducing the ratio of low-density lipoprotein
10 cholesterol to high-density lipoprotein cholesterol (LDL/HDL) by about 9.3%. For the foregoing three indexes, the proportions of respondents who improved are 88.9%, 77.8% and 77.8%, respectively.

In addition, according to FIG. 15 and Table 18, it is
15 known that after continuously taking *Bifidobacterium breve* BCRC 911145 viable bacteria capsules for 4 weeks, fasting blood glucose can be significantly reduced by 9.1%, and the proportion of respondents who improved reaches 90.0%.

From the above, it is known that *Bifidobacterium breve*
20 BCRC 911145 viable bacteria does have the effects of reducing appetite, reducing total cholesterol and low-density lipoprotein cholesterol (LDL-C), increasing high-density lipoprotein cholesterol (HDL-C) and reducing

fasting blood sugar on human body.

D-2. Efficacy test of viable bacteria composition

D-2-1. Efficacy test of composition containing viable 5 bacteria and soybean extract

Aims of the experiment: To test the weight loss effect of a composition containing *Bifidobacterium breve* BCRC 911145 viable bacteria and soybean extract.

Experimental content:

10 1. Dosage form: powder pack

2. Dosage: 4000 mg/package of a composition containing *Bifidobacterium breve* BCRC 911145 viable bacteria and soybean extract (containing 150 mg (equivalent to 2×10^8 CFU/g) of *Bifidobacterium breve* BCRC 911145
15 viable bacteria powder and 150 mg of soybean extract and additives)

3. Experimental Design: Subjects took 1 pack 1 hour before lunch daily for taking 60 consecutive days.

4. Number of persons tested: 30 persons

20 5. Conditions for inclusion of subjects: Male or female aged 20-40

6. Test items:

Body weight and body fat

The results are shown in Table 19, and FIG. 16A and FIG. 16B.

Table 19: Changes in body weight and body fat percentage of subjects

Subject number	Day 0 body weight (Initial body weight)	Day 0 body fat percentage (Initial body fat percentage)	Day 14 body weight	Day 14 body fat percentage	Day 28 body weight	Day 28 body fat percentage
1	55.7	26.3	54.6	26.8	54.6	25.6
2	58	29.6	55.4	27	55	26.7
3	73.6	26	71.6	25.1	70.5	24.9
4	79	40.3	76.1	40.6	74.4	37.8
5	97.7	35.6	95.2	35.5	94.1	38.3
6	61.9	29.4	59.5	28.4	58.9	28
7	71.8	23.4	71.7	23.9	71	23
8	70.4	41.7	68.4	40.4	67.9	41
9	69	24.1	67.7	23.9	67.6	24.2
10	71.2	36.8	69.4	35.7	68.4	34.9
11	62	36.7	60.7	36	60.3	38.7
12	50	41.2	48.2	41.6	48.2	40.5
13	88.4	32	85	32	83.6	31.2

14	84.4	31.9	81.4	30.8	79.3	30.3
15	84.4	30.5	83	29.9	82.4	27.5
16	118.6	32.9	115.2	32.9	115	32.8
17	78.3	42.4	78	42	76	40
18	75.4	38.8	72.3	36.8	72.2	36.6
19	64	32.3	60.4	30.1	60.4	31.1
20	102.9	31.8	99.9	30.8	99.9	30.9
21	61.8	38.1	60	37	60	35
22	111.5	42.6	106.7	41.7	104.1	40.8
23	53.3	28.8	51	27.9	51	28
24	84.3	33.6	83	33	82	32.5
25	81.1	24.9	77.2	24.3	75.4	23.1
26	54.1	29.1	52.6	28.7	50.8	27
27	53.2	28.6	50.2	27	49.3	25.8
28	79.8	29.5	76	27	75	26.3
29	54.2	30.7	51.6	30.1	51.3	29.4
30	78.7	42.3	77.8	41.9	77.8	41.9

Based on Table 19, and FIG. 16A and FIG. 16B, it is known that the composition containing *Bifidobacterium breve* BCRC 911145 viable bacteria and soybean extract can indeed reduce body weight and body fat percentage, and has a weight loss effect.

D-2-2. Efficacy test of composition containing viable bacteria, soybean extract and apple extract

D-2-2-1. Blood glucose modulating efficacy test of composition containing viable bacteria, soybean extract and apple extract

Mode 1

Aims of the experiment: To test the blood glucose modulating effect of a composition containing *Bifidobacterium breve* BCRC 911145 viable bacteria, soybean extract and apple extract.

Experimental content:

1. Dosage form: powder pack

2. Dosage: 800 mg/package of a composition containing *Bifidobacterium breve* BCRC 911145 viable bacteria, soybean extract and apple extract (containing 150 mg (equivalent to 5×10^9 CFU/g) of *Bifidobacterium breve* BCRC 911145 viable bacteria powder, 150 mg of soybean extract, 150 mg of apple extract and additives)

3. Number of persons tested: 5 persons

4. Conditions for inclusion of subjects: Male or female aged 20-40

5. Experimental Design:

Subject self-control experiment. For the same group of subjects, blood samples were collected at the same time intervals when the subjects did not take the composition of the present disclosure (the control group) and when the subjects took the composition of the present disclosure (the group taking the composition of the present disclosure).

The subjects took 1 pack of the above composition on Day 1 of the experiment, and did not take the composition on Day 7 of the experiment. On Day 1 of the experiment, the subjects' blood was collected when they were fasting, and then the subjects were asked to take 1 pack of the above composition, and the blood of the subjects was collected 15 minutes, 45 minutes, 60 minutes, 120 minutes and 240 minutes after taking it. After 7 days of washout, the subjects' blood was collected again when they were fasting (on Day 9 of the experiment), and then the blood of the subjects was collected at 15 minutes, 45 minutes, 60 minutes, 120 minutes and 240 minutes.

6. Test items:

- (1) Changes in serum GLP-1 concentration;
- (2) Changes in blood glucose concentration

The results are shown in FIG. 17 and FIG. 18.

FIG. 17 shows that 60 minutes after taking the

aforementioned composition, the concentration of glucagon-like peptide-1 (GLP-1) in the serum of the subjects increases significantly by 17.6%, indicating that the aforementioned composition can effectively promote the secretion of glucagon-like peptide-1 (GLP-1) in the body. In contrast, when the aforementioned composition is not taken, the concentration of glucagon-like peptide-1 (GLP-1) in the serum of the subjects fluctuated greatly. There is no trend of stable increase.

FIG. 18 shows that compared to not taking the aforementioned composition, the blood glucose level of the subjects after taking the aforementioned composition is more stable without fluctuation, indicating that taking the aforementioned composition can effectively help modulate blood glucose and achieve the effect of controlling appetite by stabilizing blood glucose.

From the above, it is known that the above composition does have the effect of modulating blood glucose and can control appetite.

Mode 2

Aims of the experiment: To test the blood glucose modulating effect of a composition containing *Bifidobacterium breve* BCRC 911145 viable bacteria,

soybean extract and apple extract.

Experimental content:

1. Dosage form: powder pack

2. Dosage: 800 mg/package of a composition containing
5 Bifidobacterium breve BCRC 911145 viable bacteria,
soybean extract and apple extract (containing 150 mg
(equivalent to 5×10^9 CFU/g) of Bifidobacterium breve
BCRC 911145 viable bacteria powder, 150 mg of soybean
extract, 150 mg of apple extract and additives)

10 3. Number of persons tested: 4 persons

4. Conditions for inclusion of subjects: Male or female
aged 20-40

5. Experimental Design:

Subject self-control experiment. For the same group of
15 subjects, blood samples were collected at the same time
intervals when the subjects did not take the composition of
the present disclosure (the control group) and when the
subjects took the composition of the present disclosure (the
group taking the composition of the present disclosure).

20 On Day 1 of the experiment, the subjects' blood was
collected when they were fasting, and then the subjects were
asked to finish breakfast (a corn Chinese omelet) within 15
minutes, and the subjects' blood was collected 2 hours and

4 hours after having breakfast. After an interval of 3 days (the Day 5 of the experiment), the subjects' blood was collected when they were fasting, and then the subjects were asked to take 1 pack of the above-mentioned composition and finish the same breakfast as before within 15 minutes, and the subjects' blood was collected 2 hours and 4 hours after having breakfast. After the subjects took the above-mentioned composition for 3 consecutive days (Day 5 to Day 7 of the experiment), the subjects' blood was collected again when they were fasting (Day 8 of the experiment).

6. Test items:

- (1) Changes in serum glucagon-like peptide-1 (GLP-1) concentration;
- (2) Changes in blood glucose concentration and changes in insulin concentration;
- (3) Fasting blood glucose concentration;
- (4) Appetite survey.

The results are shown in FIG. 19, FIG. 20A and FIG. 20B, FIG. 21, and FIG. 22A to FIG. 22C.

FIG. 19 shows that after 2 hours and 4 hours of taking the above composition, the serum glucagon-like peptide-1 (GLP-1) of the subjects continue to increase steadily, which means that taking the above composition can effectively

promote the secretion of glucagon-like peptide-1 (GLP-1) in the body and help modulate blood glucose and control appetite. In contrast, the serum glucagon-like peptide-1 (GLP-1) concentration of the control group does not
5 increase.

According to FIG. 20A and FIG. 20B, it is known that 4 hours after having breakfast, the subjects' postprandial blood glucose increases significantly. In contrast, 4 hours after having the above composition, the increase level in
10 postprandial blood glucose of the subjects is significantly lower than that of the control group, which indicates that the above composition can effectively help stabilize postprandial blood glucose.

Based on FIG. 21, it is known that after taking the
15 aforementioned composition for 3 consecutive days, the fasting blood glucose of the subjects improves, which means that the aforementioned composition can effectively help stabilize fasting blood glucose.

Furthermore, based on FIG. 22A to FIG. 22C, it is
20 known that after taking the above composition, the subjects' feeling of hunger and appetite level 4 hours after finishing breakfast both are lower than those of the control group. The subjects are less likely to feel hungry and less likely

to experience sporadic food cravings before lunch.

From the above, it is known that the above composition does have the effects of modulating blood glucose and reducing appetite.

5 Although the present disclosure has been disclosed as above with preferred embodiments, it is not intended to limit the present disclosure. Anyone skilled in the art may make some changes and modifications without departing from the spirit and scope of the present disclosure.
10 Therefore, the protection scope of the present disclosure shall be subject to the scope of the claims attached hereto.

Deposit of biological material

Domestic deposit

15 1. *Bifidobacterium breve*, *Bifidobacterium breve*
TCI761

Bioresource Collection and Research Center of Food Industry Research and Development Institute, Taiwan, China

20 August 8, 2022

BCRC 911145

Overseas deposit

1. *Bifidobacterium breve*, *Bifidobacterium breve*
TCI761

German Collection of Microorganisms and Cell Cultures
GmbH (DSMZ)

5 September 7, 2022

DSM 34383

CLAIMS

1. A novel *Bifidobacterium breve* which is deposited in the Bioresource Collection and Research Center of Food Industry Research and Development Institute, and the deposit number of which is BCRC 911145.

2. Use of the culture as claimed in claim 1 in the manufacture of a composition for reducing appetite and/or promoting glucagon-like peptide-1 (GLP-1) secretion.

3. Use of the novel *Bifidobacterium breve* as claimed in claim 1 or a culture thereof in the manufacture of a composition for inhibiting adipocyte maturation.

4. Use of the novel *Bifidobacterium breve* as claimed in claim 1 or a culture thereof in the manufacture of a composition for reducing fat accumulation and/or promoting fat metabolism.

5. Use of the novel *Bifidobacterium breve* as claimed in claim 1 or a culture thereof in the manufacture of a composition for reducing body weight.

6. Use of the novel *Bifidobacterium breve* as claimed in claim 1 or a culture thereof in the manufacture of a

composition for modulating blood lipids.

7. The use as claimed in claim 6, wherein the modulating blood lipids comprise:

reducing total cholesterol;

5 promoting high-density lipoprotein cholesterol (HDL-C) synthesis; and/or

promoting low-density lipoprotein cholesterol (LDL-C) metabolism.

8. Use of the novel *Bifidobacterium breve* as claimed in
10 claim 1 or a culture thereof in the manufacture of a composition for modulating blood glucose.

9. The use as claimed in any one of claims 2, 3, 4, 5, 6 and 8, wherein the culture comprises the novel *Bifidobacterium breve* which has been cultured.

15 10. The use as claimed in any one of claims 2, 3, 4, 5, 6 and 8, wherein the novel *Bifidobacterium breve* which has been cultured is active or a viable bacterium.

11. The use as claimed in any one of claims 2, 3, 4, 5, 6 and 8, wherein the novel *Bifidobacterium breve* which
20 has been cultured has been stripped of its own physiological activity or is a dead bacterium.

12. The use as claimed in any one of claims 2, 3, 4, 5, 6

and 8, wherein the culture comprises a cultured supernatant of the novel *Bifidobacterium breve*.

13. A probiotic composition, comprising:

the novel *Bifidobacterium breve* as claimed in claim 1 or
5 a culture thereof; and
a prebiotic.

14. The probiotic composition as claimed in claim 13,
wherein the weight ratio of the novel *Bifidobacterium
breve* or the culture thereof to the prebiotic is 1-6:1-12.

10 15. The probiotic composition as claimed in claim 13,
wherein the culture comprises the novel *Bifidobacterium
breve* which has been cultured.

16. The probiotic composition as claimed in claim 15,
wherein the novel *Bifidobacterium breve* which has been
15 cultured is active or a viable bacterium.

17. The probiotic composition as claimed in claim 15,
wherein the novel *Bifidobacterium breve* which has been
cultured has been stripped of its own physiological
activity or is a dead bacterium.

20 18. The probiotic composition as claimed in claim 13,
wherein the culture comprises a cultured supernatant of
the novel *Bifidobacterium breve*.

19. The probiotic composition as claimed in claim 13,
wherein the prebiotic comprises at least one of the
following ingredients:

a soybean extract, an oat extract, a barley extract, an
apple extract, a banana extract, a tomato extract, a
chicory extract, an asparagus extract, a beet extract, a
garlic extract, an onion extract, a seaweed extract, a
pectin extract and a tea extract.

20. The probiotic composition as claimed in claim 19,
wherein the prebiotic comprises a soybean extract.

21. The probiotic composition as claimed in claim 19,
wherein the prebiotic comprises a soybean extract and an
apple extract.

22. The probiotic composition as claimed in claim 13,
wherein the probiotic composition is a food composition.