

**USE OF CONJUGATED CHOLIC ACID TRPCA AND A STRAIN
PRODUCING THE SAME FOR TREATING AND PREVENTING A
METABOLIC DISEASE**

Priority

[0001] The application claims the priority of the Chinese application No. 202410620762.5, filed on May 17, 2024 and entitled “Use of conjugated cholic acid TrpCA and a strain producing the same for treating and preventing a metabolic disease”, the full content of which is incorporated by reference.

TECHNICAL FIELD

[0002] The disclosure relates to the field of microbiology. In particular, the disclosure relates to the use of a conjugated cholic acid TrpCA and the strain producing the same for treating and preventing metabolic-related diseases.

BACKGROUND

[0003] Metabolic diseases are a general term for diseases that occur due to metabolic disorders in the body. Metabolic diseases are usually caused by an imbalance of carbohydrates, lipids, proteins, vitamins, electrolytes, water, etc. Examples of metabolic diseases include obesity, diabetes, hyperlipidemia, arteriosclerosis, hypertension, non-alcoholic fatty liver disease, and the like. With the development of society, the continuous improvement of people's living standards and the adjustment of dietary structure, the trend of dietary surplus has led to the increasing incidence of metabolic diseases, among which obesity, hyperglycemia and hyperlipidemia are the most attractive. Obesity is an inducer of a range of diseases, such as hypertension, diabetes, coronary heart disease, gallbladder disease, osteoarthritis, and respiratory distress during sleep. Drugs for losing weight, reducing blood sugar and decreasing blood lipids have become one of the focuses of drug research.

[0004] There remains a need in the art for active ingredients capable of preventing and treating metabolic diseases.

SUMMARY

[0005] Tryptophan-conjugated cholic acid (also known as TrpCA or TRP-CA) is a newly discovered amino acid-conjugated cholic acid produced by bacteria in recent years, which is widely present in the population. However, its physiological function has not been reported yet. The research results of the present inventor indicate that the tryptophan-conjugated cholic acid can significantly promote the secretion of GLP-1 in both *in vivo* animal model and *in vitro* cell model, thus play a role in reducing weight, lowering blood sugar and relieving the symptoms of non-alcoholic fatty liver disease. Furthermore, the colonization of probiotics capable of producing TrpCA can also play a similar role in improving metabolism.

[0006] Through extensive and in-depth research and experiments, the present inventor found that the tryptophan-conjugated cholic acid has the function of preventing and treating metabolism-related diseases (including obesity, diabetes and fatty liver). Further, strains that can produce tryptophan-conjugated cholic acid were screened through *in vitro* experiments. And *Bifidobacterium animalis subspecies lactis* (*B. animalis subsp. lactis*), which can produce the tryptophan-conjugated cholic acid, was colonized in mice fed with high-fat diet and the strain. It is found that the colonization of the strain could also reduce blood sugar and effectively alleviate diabetes by producing the tryptophan-conjugated cholic acid. On this basis, the present disclosure is completed.

[0007] In an aspect, provided herein is use of a tryptophan-conjugated cholic acid or a strain producing the same in the preparation of a pharmaceutical composition or food composition, wherein the pharmaceutical composition is used for preventing and/or treating a metabolic disease or for promoting GLP-1

and/or insulin production, and the food-composition is used for maintaining healthy blood glucose level, maintaining healthy blood lipids and/or regulating body fat.

[0008] In an aspect, provided herein is a method for preventing and/or treating a metabolic disease or for promoting GLP-1 and/or insulin production in subjects, comprising administering tryptophan-conjugated cholic acid or a strain producing the same or a pharmaceutical composition comprising tryptophan-conjugated cholic acid or strain producing the same, to the subjects.

[0009] In an aspect, provided herein is a method for maintaining healthy blood glucose level, maintaining healthy blood lipids and/or controlling body fat in subjects, comprising administering tryptophan-conjugated cholic acid or a strain producing the same or a food composition comprising the tryptophan-conjugated cholic acid or strains producing the same, to the subjects.

[0010] In an aspect, provided herein is tryptophan-conjugated cholic acid or a strain producing the same or a pharmaceutical composition comprising the tryptophan-conjugated cholic acid or strain producing the same for use in preventing and/or treating a metabolic disease or for promoting GLP-1 and/or insulin production.

[0011] In an aspect, provided herein is a tryptophan-conjugated cholic acid or a strain producing the same or a food-composition comprising the tryptophan-conjugated cholic acid or strain producing the same for use in maintaining healthy blood glucose level, maintaining healthy blood lipids and/or controlling body fat.

[0012] In an embodiment of the above aspects, the subject is a mammal, even more preferably a human being.

[0013] In an embodiment of the above aspects, the strain producing the tryptophan-conjugated cholic acid is one or more of the following genera: *Streptococcus*, *Pediococcus*, *Lactobacillus*, *Limosilactobacillus*,

Lactilactobacillus, *Ligilactobacillus*, *Lactiplantibacillus*, *Bifidobacterium*, and *Lactococcus*.

[0014] In an embodiment of the above aspects, the strain producing the tryptophan-conjugated cholic acid is one or more of the following species: *Streptococcus salivarius*, *Pediococcus acidilactici*, *Lactobacillus gasseri*, *Limosilactobacillus reuteri*, *Lactilactobacillus sakei*, *Lactobacillus acidophilus*, *Ligilactobacillus salivarius*, *Lactiplantibacillus plantarum*, *Bifidobacterium animalis* and *Lactococcus lactis*.

[0015] In an embodiment of the above aspects, *Streptococcus salivarius* is *Streptococcus salivarius subsp. thermophilus*. In an embodiment of the above aspects, *Bifidobacterium animalis* is *Bifidobacterium animalis subsp. lactis*.

[0016] In an embodiment of the above aspects, the metabolic disease is GLP-1 related metabolic diseases, preferably selected from diabetes or diabetes related diseases.

[0017] In an embodiment of the above aspects, diabetes is selected from type 1 diabetes, type 2 diabetes, gestational diabetes, obesity diabetes, autoimmune diabetes and borderline diabetes.

[0018] In an embodiment of the above aspects, diseases are related to diabetes are selected from obesity, an obesity-related disorder, metabolic syndrome, diabetic neuropathy, kidney disease such as diabetic nephropathy, diabetic retinopathy, diabetic cardiomyopathy, cataract, macroangiopathy, osteopenia, hyperosmolar diabetic coma, diabetic gangrene, xerostomia, hypoacusis, diabetic cachexia, delayed wound healing, diabetic dyslipidemia, peripheral blood circulation disorder, non-alcoholic steatohepatitis, pre-diabetes, hyperlipemia, fatty liver diseases, impaired fasting glucose, hyperglycemia, impaired glucose tolerance, insulin resistance, hyperinsulinemia, elevated blood fatty acids or glycerol levels, impaired wound healing, glucose intolerance, increased fasting glucose and dyslipidemia, insufficient numbers of pancreatic beta-cells,

enteroendocrine cell insufficiency, glycosuria, metabolic acidosis, cataract, diabetic coronary heart disease, diabetic cerebrovascular disease, diabetic peripheral vascular disease, atherosclerosis and stroke.

[0019] In an embodiment of the above aspects, the obesity is selected from symptomatic obesity, simple obesity, childhood obesity, morbid obesity and abdominal obesity.

[0020] In an embodiment of the above aspects, obesity related disease is selected from impaired glucose tolerance, abnormal lipid metabolism, hyperlipidemia, hypertension, heart failure, hyperuricemia, gout, fatty liver, coronary heart disease, cerebral infarction, sleep apnea syndrome, obesity hypoventilation syndrome and visceral obesity syndrome.

[0021] In an embodiment of the above aspects, the food composition further comprises food-acceptable carriers, diluents or excipients.

[0022] In an embodiment of the above aspects, the pharmaceutical composition further comprises pharmaceutically acceptable carriers, diluents or excipients.

[0023] In an embodiment of the above aspects, the food composition or pharmaceutical composition further comprises probiotics, and/or prebiotics.

[0024] In an embodiment of the above aspects, the composition is an oral composition, preferably selected from a group consisting of enteric coated tablets, granules, capsules, tablets, powders, oral liquids, suspensions and emulsions.

[0025] In a further aspect of the above aspects, provided herein is a composition for preventing and/or treating a metabolic disease, comprising a tryptophan-conjugated cholic acid or a strain producing the same and further comprising another active ingredient for preventing and/or treating a metabolic disease. In an embodiment, the another active ingredient is one or more of biguanides, sulfonylureas, glinides, an alpha-glucosidase inhibitors, thiazolidinediones and GLP-1 drugs. In an embodiment, the composition is an

oral composition. In an embodiment, the oral composition is selected from a group consisting of enteric coated tablets, granules, capsules, tablets, powders, oral liquids, suspensions and emulsions.

[0026] In an embodiment of the above aspects, the food composition further comprises food acceptable carriers, diluents or excipients.

[0027] In an embodiment of the above aspects, the pharmaceutical composition further comprises pharmaceutically acceptable carriers, diluents or excipients.

[0028] In an embodiment of the above aspects, the food composition or pharmaceutical composition further comprises probiotics, and/or prebiotics.

[0029] In an embodiment of the above aspects, the composition is an oral composition, preferably selected from a group consisting of granules, capsules, tablets, powders, oral liquids, suspensions and emulsions. In an embodiment, the composition is administrated at 1×10^{-1} - 1×10^{12} CFU/mL, 1×10^4 - 1×10^9 CFU/mL or 1×10^5 - 1×10^8 CFU/mL of the strain producing the tryptophan-conjugated cholic acid.

[0030] In an aspect, provided herein is a method for producing tryptophan-conjugated cholic acid, comprising culturing probiotics in the presence of bile acids and tryptophan, wherein the probiotics belongs to one or more of the following genera: *Streptococcus*, *Pediococcus*, *Lactobacillus*, *Limosilactobacillus*, *Lactilactobacillus*, *Ligilactobacillus*, *Lactiplantibacillus*, *Bifidobacterium*, and *Lactococcus*.

[0031] In an embodiment, the probiotics from one or more of the following species: *Streptococcus salivarius*, *Pediococcus acidilactici*, *Lactobacillus gasseri*, *Limosilactobacillus reuteri*, *Lactilactobacillus sakei*, *Lactobacillus acidophilus*, *Ligilactobacillus salivarius*, *Lactiplantibacillus plantarum*, *Bifidobacterium animalis* and *Lactococcus lactis*.

[0032] In an embodiment, *Streptococcus salivarius* is *Streptococcus salivarius*

subsp. thermophilus. In another embodiment, *Bifidobacterium animalis* is *Bifidobacterium animalis subsp. lactis*.

[0033] In an aspect, provided herein is use of probiotics for the production of a tryptophan-conjugated cholic acid, wherein the probiotic is one or more of the following genera: *Streptococcus*, *Pediococcus*, *Lactobacillus*, *Limosilactobacillus*, *Lactilactobacillus*, *Ligilactobacillus*, *Lactiplantibacillus*, *Bifidobacterium*, and *Lactococcus*.

[0034] In an embodiment, the probiotic is one or more of following species: *Streptococcus salivarius*, *Pediococcus acidilactici*, *Lactobacillus gasseri*, *Limosilactobacillus reuteri*, *Lactilactobacillus sakei*, *Lactobacillus acidophilus*, *Ligilactobacillus salivarius*, *Lactiplantibacillus plantarum*, *Bifidobacterium animalis* and *Lactococcus lactis*.

[0035] In an embodiment, *Streptococcus salivarius* is *Streptococcus salivarius subsp. thermophilus*. In another embodiment, *Bifidobacterium animalis* is *Bifidobacterium animalis subsp. lactis*.

[0036] In an aspect, provided herein is a method for screening probiotics that promote GLP-1 and/or insulin production, comprising:

[0037] (1) culturing probiotics in the presence of bile acids and tryptophan to obtain culture supernatant;

[0038] (2) measuring the content of the tryptophan-conjugated cholic acid in the culture supernatant; and

[0039] (3) selecting the probiotics that produces the tryptophan-conjugated cholic acid.

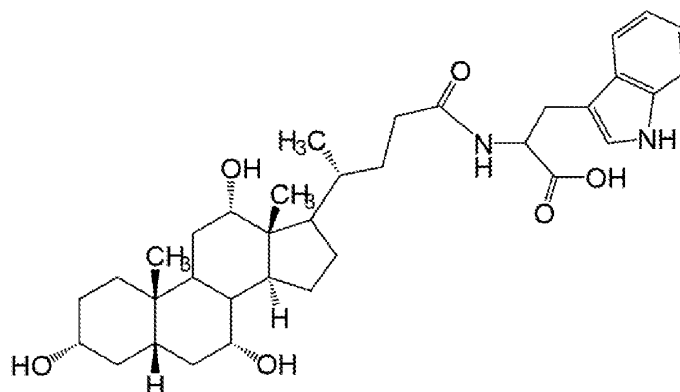
[0040] In an embodiment, the probiotics belongs to one or more of the following genera: *Streptococcus*, *Pediococcus*, *Lactobacillus*, *Limosilactobacillus*, *Lactilactobacillus*, *Ligilactobacillus*, *Lactiplantibacillus*, *Bifidobacterium*, and *Lactococcus*.

[0041] In an embodiment, the probiotics belongs to a species selected from one or more of *Streptococcus salivarius*, *Pediococcus acidilactici*, *Lactobacillus gasseri*, *Limosilactobacillus reuteri*, *Lactilactobacillus sakei*, *Lactobacillus acidophilus*, *Ligilactobacillus salivarius*, *Lactiplantibacillus plantarum*, *Bifidobacterium animalis* and *Lactococcus lactis*.

[0042] In an embodiment, *Streptococcus salivarius* is *Streptococcus salivarius* subsp. *thermophilus*. In an embodiment, *Bifidobacterium animalis* is *Bifidobacterium animalis* subsp. *lactis*.

[0043] In an embodiment, the method comprises mixing the culture medium of probiotics with methanol and detecting the content of the tryptophan-conjugated cholic acid by mass spectrometry.

[0044] In an embodiment, the tryptophan-conjugated cholic acid has the following structure:



[0045]

[0046] Advantages of the present disclosure include:

[0047] 1. The inventors discover for the first time that tryptophan-conjugated cholic acid can significantly promote the secretion of GLP-1 in both *in vivo* animal model and *in vitro* cell model, thereby reducing weight, lowering blood sugar, and relieving symptoms of non-alcoholic fatty liver disease.

[0048] 2. The inventors provide the method for screening probiotics that promotes GLP-1 and/or insulin production according to the production of the

tryptophan-conjugated cholic acid.

[0049] 3. The inventors discover that the genus *Bifidobacterium* (e.g., *Bifidobacterium animalis*) can produce tryptophan-conjugated cholic acid in high yields.

DESCRIPTION OF THE DRAWINGS

[0050] Fig. 1 shows the structural formula of the tryptophan-conjugated cholic acid.

[0051] Fig. 2 shows the effect of the tryptophan-conjugated cholic acid on body weight in high-fat fed mice. The statistical method was a two-way ANOVA on duplicate measurements with unpaired T test. * $P < 0.05$, ** $P < 0.01$.

[0052] Fig. 3 shows the effect of the tryptophan-conjugated cholic acid on glucose tolerance in high-fat fed mice. The upper panel of Fig. 3 shows the change in blood glucose level over time. The lower panel of Fig. 3 shows the AOC (area of the curve) of the control and treatment groups. The statistical method was two-way ANOVA on duplicate measurements with unpaired T test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[0053] Fig. 4 shows the effect of the tryptophan-conjugated cholic acid on serum AST and ALT in high-fat fed mice. The statistical method was a two-tailed unpaired T test. * $P < 0.05$, *** $P < 0.001$.

[0054] Fig. 5 shows the change in the content of the tryptophan-conjugated cholic acid in the ileum of mice after one day of gavage tryptophan-conjugated cholic acid (10 mg/kg) to mice for 1 week. The statistical method was a two-tailed unpaired T test. *** $P < 0.001$.

[0055] Fig. 6 shows the effect of *Bifidobacterium animalis subsp. lactis* on glucose tolerance in high-fat fed mice. The statistical method was two-way ANOVA on duplicate measurements with unpaired T test. * $P < 0.05$, ** $P < 0.01$. The upper panel of Fig. 6 shows the changes in blood glucose level over time.

The lower panel of Fig. 6 shows the AOC (area of the curve) of the control and treatment groups.

DETAILED DESCRIPTION

[0056] As used herein, the term “tryptophan-conjugated cholic acid” refers to a conjugate formed by the reaction of the amino group of tryptophan with the carboxyl group at position 24 of a bile acid to form an amide bond. The structure of tryptophan-conjugated cholic acid is shown in Fig. 1. Tryptophan-conjugated cholic acid has been reported, for example, in Nature. 2024 Feb; 626(7998):419-426. doi: 10.1038/s41586-023-06906-8. Epub 2023 Dec 5.

[0057] As used herein, the term “a strain producing the tryptophan-conjugated cholic acid” refers to probiotics which is capable of producing the tryptophan-conjugated cholic acid in the presence of tryptophan and bile acids, which are substrates for the tryptophan-conjugated cholic acid. Examples of the strain producing the tryptophan-conjugated cholic acid include, but are not limited to, strains which belong to a genus selected from *Streptococcus*, *Pediococcus*, *Lactobacillus*, *Limosilactobacillus*, *Lactilactobacillus*, *Ligilactobacillus*, *Lactiplantibacillus*, *Bifidobacterium*, and *Lactococcus*. The strain producing the tryptophan-conjugated cholic acid may belong to a species selected from one or more of *Streptococcus salivarius*, *Pediococcus acidilactici*, *Lactobacillus gasseri*, *Limosilactobacillus reuteri*, *Lactilactobacillus sakei*, *Lactobacillus acidophilus*, *Ligilactobacillus salivarius*, *Lactiplantibacillus plantarum*, *Bifidobacterium animalis* and *Lactococcus lactis*. More specifically, *Streptococcus salivarius* may be *Streptococcus salivarius* subsp. *thermophilus*. *Bifidobacterium animalis* may be *Bifidobacterium animalis* subsp. *lactis*.

[0058] As used herein, the term “glucagon-like peptide-1 (GLP-1)” is a hormone mainly produced by intestinal L cells. GLP-1 exerts its hypoglycemic effect by promoting insulin secretion, and can also reduce weight and blood lipids by inhibiting appetite and promoting energy metabolism.

[0059] As used herein, the term “metabolic disease” generally refer to a disease or condition that disrupts the normal metabolic process in the body, making it impossible for the body to utilize and/or store energy normally. Metabolic diseases can be any metabolic disease that benefits from modulating (e.g., increasing) GLP-1 activity, or from stimulating GLP-1 receptor activity. Metabolic diseases may be a GLP-1 related metabolic diseases, including diabetes or diabetes related diseases. Diabetes includes but is not limited to type 1 diabetes, type 2 diabetes, gestational diabetes, obesity diabetes, autoimmune diabetes and/or borderline diabetes. Diseases related to diabetes include but are not limited to obesity, obesity-related disorder, metabolic syndrome, diabetic neuropathy, kidney diseases such as diabetic nephropathy, diabetic retinopathy, diabetic cardiomyopathy, cataract, macroangiopathy, osteopenia, hyperosmolar diabetic coma, diabetic gangrene, xerostomia, hypoacusis, diabetic cachexia, delayed wound healing, diabetic dyslipidemia, peripheral blood circulation disorders, non-alcoholic steatohepatitis, pre-diabetes, hyperlipemia, fatty liver disease, impaired fasting glucose, hyperglycemia, impaired glucose tolerance, insulin resistance, hyperinsulinemia, elevated blood fatty acids or glycerol levels, impaired wound healing, glucose intolerance, increased fasting glucose and dyslipidemia, insufficient numbers of pancreatic beta-cells, enteroendocrine cell insufficiency, glycosuria, metabolic acidosis, cataract, diabetic coronary heart disease, diabetic cerebrovascular disease, diabetic peripheral vascular disease, atherosclerosis and/or stroke. Obesity includes, but is not limited to, symptomatic obesity, simple obesity, childhood obesity, morbid obesity and abdominal obesity. Obesity related diseases include, but are not limited to, impaired glucose tolerance, abnormal lipid metabolism, hyperlipidemia, hypertension, heart failure, hyperuricemia, gout, fatty liver, coronary heart disease, cerebral infarction, sleep apnea syndrome, obesity hypoventilation syndrome and visceral obesity syndrome.

[0060] As used herein, the term “prevention” or “preventing” refers to the

delayed onset, and/or a reduction in the frequency and/or severity of one or more symptoms of a particular disease, condition or illness. The term “treatment” or “treating” refers to any administration of a therapy that partially or completely alleviates, ameliorates, mitigates, inhibits one or more symptoms, features and/or causes of a particular disease, condition and/or illness, delays its onset, reduces its severity, and/or reduces its incidence rate.

[0061] As used herein, the term “subject” refers to an individual who receives the administration treatment. In some embodiments, the subject is an animal. In some embodiments, the subject is a mammal, e.g., a mammal that experiences or is susceptible to metabolic diseases as described herein. In some embodiments, the animal is a vertebrate, e.g., a mammal, such as a non-human primate (particularly a higher primate), sheep, dog, rodent (e.g., mouse or rat), guinea pig, goat, pig, cat, rabbit, or cow. In some embodiments, the subject is a human.

[0062] As used herein, “pharmaceutically effective dosage” means an amount sufficient to treat or prevent a disease at a reasonable benefit-risk ratio suitable for medical treatment or prevention. And the effective dosage level may be determined based on factors including disease severity, drug activity, age, weight, health status, gender of the patients, sensitivity to the drugs, and time, route, release rate, duration of administration, drugs used in combination or concurrently with the combination, and other factors known in the medical arts. According to one embodiment, a pharmaceutical composition may be administered as a therapeutic agent alone, or in combination with other therapeutic agents, and can be administered sequentially or concurrently with conventional therapeutic agents. In addition, it can be administered singly or multiple times. Taking all factors into consideration, it is important to administer the minimum amount to achieve the maximum efficacy without side effects.

[0063] Treatment method

[0064] The present disclosure provides use of the tryptophan-conjugated cholic acid in the treatment and prevention of metabolic-related diseases.

Tryptophan-conjugated cholic acid has the ability to inhibit weight gain in a subject ingested a high-fat diet. The inventors found that after the treatment of the tryptophan-conjugated cholic acid, C57BL/6J mice fed with a high-fat diet, which can lead to obesity, gained less weight and obtained the improvement of blood glucose and fatty liver symptoms compared to untreated controls. Therefore, it is concluded that the tryptophan-conjugated cholic acid can be used for preventing and treating obesity and diseases caused by the obesity, such as diabetes, fatty liver and the like. The present disclosure also provides use of tryptophan-conjugated cholic acid producing bacteria in the treatment and prevention of metabolic related diseases. The following genera were tested *in vitro*, including *Streptococcus*, *Pediococcus*, *Lactobacillus*, *Limosilactobacillus*, *Lactilactobacillus*, *Ligilactobacillus*, *Lactiplantibacillus*, *Bifidobacterium*, and *Lactococcus*, all of which can produce tryptophan-conjugated cholic acid. Therefore, technicians in this field can expect that these strains tested *in vitro* can improve the glycolysis metabolism disorders induced by a high-fat diet. The inventors also demonstrate in a mouse model that colonization of *Bifidobacterium animalis subsp. lactis* significantly ameliorates glycolysis metabolism disorders induced by high-fat diet.

[0065] Accordingly, the present disclosure provides a method for treating and/or preventing a metabolic disease in a subject comprising administering the tryptophan-conjugated cholic acid or a strain producing the same to the subject. The present disclosure also provides a method for promoting the production of GLP-1 and/or insulin in a subject comprising administering the cholic acid conjugated to tryptophan or a strain producing the same to the subject.

[0066] The type of the strain producing a tryptophan-conjugated cholic acid is not particularly limited, as long as it is probiotics capable of producing the tryptophan-conjugated cholic acid. The strain producing a tryptophan-conjugated cholic acid may include the strains in the genus *Streptococcus*, *Pediococcus*, *Lactobacillus*, *Limosilactobacillus*, *Lactilactobacillus*, *Ligilactobacillus*,

Lactiplantibacillus, *Bifidobacterium*, and/or *Lactococcus*. For example, the strain producing tryptophan-conjugated cholic acid may be one or more species of *Streptococcus salivarius*, *Pediococcus acidilactici*, *Lactobacillus gasseri*, *Limosilactobacillus reuteri*, *Lactilactobacillus sakei*, *Lactobacillus acidophilus*, *Ligilactobacillus salivarius*, *Lactiplantibacillus plantarum*, *Bifidobacterium animalis* and *Lactococcus lactis*. *Streptococcus salivarius* may be *Streptococcus salivarius* subsp. *thermophilus*. *Bifidobacterium animalis* may be *Bifidobacterium animalis* subsp. *lactis*. A person skilled in the art can easily select a strain producing a tryptophan-conjugated cholic acid suitable for administration to a mammal, particularly a human being, based on the teachings of the present disclosure.

[0067] The methods of the present disclosure may be suitable for any mammalian subject, but is preferably suitable for human subjects. As described above, the metabolic diseases treated by the present method are those related to GLP-1 or obesity, preferably selected from diabetes or diabetes related diseases, such as those described herein.

[0068] Healthcare methods

[0069] The inventors discover that the tryptophan-conjugated cholic acid or the strain producing the same or a composition comprising the tryptophan-conjugated cholic acid or the strain producing the same can be used for maintaining healthy blood glucose level, maintaining healthy blood lipids and/or controlling body fat. The present disclosure provides a method for maintaining healthy blood glucose level, maintaining healthy blood lipids and/or controlling body fat in a subject, comprising administering to the subject the tryptophan-conjugated cholic acid or the strain producing the same or the composition comprising the tryptophan-conjugated cholic acid or the strain producing the same. In the healthcare method herein, the subject may be a healthy subject.

[0070] The type of the strain producing tryptophan-conjugated cholic acid is

not particularly limited, as long as they are probiotics that can produce tryptophan-conjugated cholic acid. The tryptophan-conjugated cholic acid producing bacteria is one or more of the genus *Streptococcus*, *Pediococcus*, *Lactobacillus*, *Limosilactobacillus*, *Lactilactobacillus*, *Ligilactobacillus*, *Lactiplantibacillus*, *Bifidobacterium*, and/or *Lactococcus*. For example, the tryptophan-conjugated cholic acid producing bacteria may be one or more of the following species: *Streptococcus salivarius*, *Pediococcus acidilactici*, *Lactobacillus gasseri*, *Limosilactobacillus reuteri*, *Lactilactobacillus sakei*, *Lactobacillus acidophilus*, *Ligilactobacillus salivarius*, *Lactiplantibacillus plantarum*, *Bifidobacterium animalis* and *Lactococcus lactis*. *Streptococcus salivarius* may be *Streptococcus salivarius subsp. thermophilus*. *Bifidobacterium animalis* may be *Bifidobacterium animalis subsp. lactis*. A person skilled in the art can easily select a producing strain of tryptophan-conjugated cholic acid suitable for administration to a mammal, particularly a human, based on the teachings of the present disclosure.

[0071] The health care method of the present disclosure can be suitable for any mammalian subject, but is preferably suitable for human subjects. These subjects need to maintain healthy blood glucose level, maintain healthy blood lipids, and/or control body fat.

[0072] Composition

[0073] The composition administered to the subjects may be a food composition such as, but not limited to, beverage, dairy products, solid foods. The food composition may further comprise additional probiotics, prebiotics, and/or food acceptable carriers, diluents, or excipients. The composition administered to a subject may also be a pharmaceutical composition. The pharmaceutical composition can further comprise another active ingredient for preventing and/or treating metabolic diseases. Preferably, the other active ingredient is one or more of biguanides, sulfonylureas, glinides, alpha-glucosidase inhibitors, thiazolidinediones and GLP-1 drugs. The

composition herein may be an oral composition, preferably selected from granules, capsules, tablets, powders, oral liquids, suspensions, and emulsions. The amount of the strain producing the tryptophan-conjugated cholic acid in the composition should be a therapeutically or prophylactically effective amount. Technicians in this field can conventionally determine the effective dosage of probiotics, such as the strain producing a tryptophan-conjugated cholic acid. For example, the unit dose of the strain producing a tryptophan-conjugated cholic acid in the composition comprises 1×10^{-1} – 1×10^{12} CFU/mL, 1×10^4 – 1×10^9 CFU/mL, or 1×10^5 – 1×10^8 CFU/mL of the strain producing a tryptophan-conjugated cholic acid.

[0074] Some non-limiting examples of food products to be used together with the methods and compositions described herein include: ice-cream stick, cheese, cream, chocolate, milk, and the like. In other embodiments, the food product may be fruit juices, refreshing drinks, tea drinks, drink products, jelly drinks and functional drinks; alcoholic beverages such as beer; carbohydrate-containing foods such as rice foods, noodles, bread and cooked wheaten food; pasty products such as fish, ham, sausage, seafood pasty product; steamed bagged product such as curry, thick starch-coated food, and Chinese soup; soup; dairy products such as milk, dairy drink, ice cream, and yogurt; fermented products such as fermented soy milk, fermented drink, and pickle; bean products; various confectionery products, including biscuit, cookie, etc., candy, gum, fudge, cold dessert, including jelly, cream caramel, and frozen dessert; instant food such as instant soup and instant bean soup; and the like. In one embodiment, the food product for administration is frozen. In some embodiments, the food product may be or comprise one or more of bars, candies, baked goods, cereals, savory snacks, cooked wheaten foods, chocolates and other solid foods, as well as liquids or semi-solid foods (including yogurt, soups, and stews) and drinks (such as smoothies, shakes, juices, and other carbonated or non-carbonated drinks).

[0075] The oral composition may comprise an inert diluent or an edible carrier.

For oral therapeutic administration, the active compounds may be blended with excipients and used in the form of tablets, lozenges, pastilles, troches, or capsules (e.g., gelatin capsules). The oral composition may also be prepared by combining the composition of the present disclosure with food. In some embodiments, the probiotics may be formulated in the food product.

[0076] In some embodiments, the composition is administered to the subject in a single dose. In some embodiments, the composition is administered to the subject in multiple doses. In some embodiments, the dosage of the composition is administered to the subject twice daily, daily, weekly, or monthly.

[0077] In some embodiments, the unit dose comprises from 10 to 10^{15} , from 10^2 to 10^{14} , from 10^3 to 10^{13} , from 10^4 to 10^{12} , from 10^5 to 10^{11} , from 10^6 to 10^{10} , from 10^2 to 10^7 , from 10^2 to 10^8 , or from 10^2 to 10^9 colony forming units (CFU). In some embodiments, the probiotics may be a viable cell. In some embodiments, the composition comprises cell culture of probiotics and/or culture supernatant thereof and/or a powder formed thereof, and/or the composition is formulated by using cell culture of probiotics and/or its culture supernatant thereof and/or powder formed thereof.

[0078] The pharmaceutical composition is generally formulated to be compatible with their intended route of administration. Examples of routes of administration include oral administration. Methods of formulating suitable pharmaceutical compositions are known in the art, see, for example, Remington: The Science and Practice of Pharmacy, 21st Edition, 2005; and books in the Drugs and the Pharmaceutical Sciences: a Series of Textbooks and Monographs (Dekker, NY) series. The oral composition typically comprises an inert diluent or an edible carrier. For example, an oral formulation may be or comprise a syrup, liquid, tablet, lozenge, fondant, capsule (e.g., gelatin capsule), powder, gel, film, or the like.

[0079] Lactose, glucose, sucrose, sorbitol, mannose, starch, acacia, calcium phosphate, alginate, gelatin, calcium silicate, fine crystalline cellulose,

polyvinylpyrrolidone (PVP), cellulose, water, syrup, methylcellulose, methylparaben, propylparaben, talc, magnesium stearate, mineral oil, or the like may be used as a carrier, excipient, diluent, or the like for the pharmaceutical composition of the present disclosure.

[0080] In addition, the pharmaceutical composition of the present disclosure may further comprise a lubricant, a wetting agent, an emulsifier, a suspoemulsion stabilizer, a preservative, a sweetener, a flavor, and the like. The pharmaceutical composition of the present disclosure can be produced in an enteric-coated formulation by a variety of well-known methods so that the microorganisms, which are the active ingredients of the pharmaceutical composition, can smoothly pass through the stomach without being destroyed by gastric acid.

[0081] The pharmaceutical composition of the present disclosure can be made into enteric coated tablets for oral administration. The term “enteric coating” in the present application includes all conventional medicinally acceptable coatings which are not degraded by gastric acid but are sufficiently broken down in the small intestine to rapidly release the microorganisms of the present disclosure. The enteric-coating of the present disclosure can be maintained for more than 2 hours in synthetic gastric acid, such as HCl solution at pH = 1, at 36-38°C, and is preferably decomposed within 1.0 hour in synthetic intestinal fluid, such as buffer solution at pH = 7.0.

[0082] The enteric coating of the present disclosure is coated at about 16-30 mg per tablet, preferably 16-25 mg, more preferably 16-20 mg. In the present disclosure, the thickness of the enteric coating is 5-100 μm , and the ideal thickness is 20-80 μm . The component of the enteric coating may be prepared using conventional polymers known in the art.

EXAMPLES

[0083] The experimental methods used in the following examples are conventional unless otherwise specified.

[0084] Materials, reagents, etc. used in the following examples are commercially available unless otherwise specified.

[0085] Example 1. Effect of tryptophan-conjugated cholic acid on glucose tolerance-related indicators, body weight, body fat, and liver function in obesity model mice

[0086] Materials: blood glucose test paper (commercially available from Roche), blood glucose meter (commercially available from Roche), active GLP-1 detection kit (commercially available from Merck), insulin detection kit (commercially available from CUSABIO), serum aspartate aminotransferase AST assay kit (commercially available from BioSino), serum alanine aminotransferase ALT assay kit (commercially available from BioSino).

[0087] Establishment of the mouse model: C57BL/6 mice were purchased from GemPharmatech, maintained at a temperature of 20-24°C, with a constant humidity of 50-60%, and exposed to light for 12 hours from 8:00 to 20:00 with soundproofing and with ad libitum feeding and drinking water. The mice were acclimated for one week before the experiment. C57BL/6 mice were fed with a high-fat diet containing the tryptophan-conjugated cholic acid for 8 weeks by gavage at a dose of 10 mg/kg body weight and a frequency of three times per week. A mouse obesity model induced by a high-fat diet in mice was established.

[0088] Methods: C57 male mice, aged 6-8 weeks, were fed with a high-fat diet and weighed 20-22g. The mice were divided into 2 groups according to their body weight: (1) control group: saline containing 1% DMSO, without tryptophan-conjugated cholic acid; (2) tryptophan-conjugated cholic acid treatment group (experimental group): 6 mice in each group received 10 mg/kg of the drug three times a week for 8 weeks. Six additional mice were used for the detection of intestinal contents in the mice. One week after intragastric administration of 10 mg/kg tryptophan-conjugated cholic acid, the mice were sacrificed, the small intestine segments were clipped and the contents were removed. The small intestinal contents of mice were extracted with 20-fold

methanol, and the concentration of tryptophan-conjugated cholic acid in the small intestinal contents of mice was detected by mass spectrometry.

[0089] The same amount of saline was given to both groups. The two groups of mice were fed with a high-fat diet for 8 weeks, and the body weight was monitored every week. Glucose tolerance test was carried out at the second week of treatment. That is, the mice received glucose by gavage, and the GLP-1 and insulin levels of mice were detected at different time points. In Table 2, GLP-1 and insulin secretion levels were measured at 0 min (i.e., before glucose administration) and at 15 min (i.e., 15 min after glucose administration). Blood glucose of the mice was measured using a blood glucose meter at 0, 15, 30, 60, 90, and 120 min. GLP-1 and insulin secretion levels were detected with active GLP-1 detection kit (Merck) and insulin detection kit (CUSABIO) according to the instructions of the kits. After the last administration (week 8), body fat was detected by nuclear magnetic resonance to determine fat content and muscle content. Blood was taken and centrifuged at 3000 rpm at 4°C. Serum aspartate aminotransferase (also known as glutamic oxalacetic transaminase) AST assay kit (BioSino) and serum glutamic-pyruvic transaminase (*viz* alanine aminotransferase) ALT assay kit (BioSin) were used to determine serum ALT and AST according to the instructions of the kit.

[0090] Results: Compared with the control group, the tryptophan-conjugated cholic acid treatment group had a trend of change from the first week, and there was a significant difference in the sixth week. These results indicated that tryptophan-conjugated cholic acid had a significant impact on body weight control and could significantly reduce body fat; and can also improve glucose tolerance induced by a high-fat diet. The improvement of glucose tolerance is due to the promotion of the secretion of glucose-stimulated GLP-1 and insulin. The tryptophan-conjugated cholic acid reduced the levels of serum AST and ALT, which are key indexes of liver function. The results are shown in Figs. 2-4 and Tables 1-2.

[0091] Fig. 2 shows the effect of the tryptophan-conjugated cholic acid on body weight of mice fed with a high-fat diet. The statistical method was a two-way ANOVA on duplicate measurements with unpaired T test. * $P < 0.05$, ** $P < 0.01$.

[0092] Fig. 3 shows the effect of the tryptophan-conjugated cholic acid on glucose tolerance of mice fed with a high-fat diet. The upper panel of Fig. 3 shows the change in blood glucose level over time. The lower panel of Fig. 3 shows the blood glucose AOC (area of the curve) of the control and treatment groups. The statistical method was a two-way ANOVA on duplicate measurements with unpaired T test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[0093] Fig. 4 shows the effect of tryptophan-conjugated cholic acid on serum AST and ALT of mice fed with a high-fat diet. The statistical method was a two-tailed unpaired T test.

[0094] Fig. 5 shows the concentration of the tryptophan-conjugated cholic acid in the ileal contents after intragastric administration of 10 mg/kg tryptophan-conjugated cholic acid to the mice for one week.

[0095] Table 1 Effect of the tryptophan-conjugated cholic acid on body fat in mice fed with a high fat diet after 8 weeks of continuous administration (The statistical method is a two-tailed unpaired T test.)

Group	Fat content (g)	Muscle content (g)
Control group	10.1	19.3
Experimental group	5.5	19.5
P value	0.001	0.800

[0096] Table 2 Effect of the tryptophan-conjugated cholic acid on glucose-stimulated GLP-1 and insulin in mice fed with a high-fat diet via glucose tolerance test in 2 weeks after continuous administration (The statistical method was a two-way ANOVA on duplicate measurements with unpaired T test.)

Group	GLP-1 (pM)	Insulin, in ng/mL
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	0 minute	15 min	0 minute	15 min
Control group	4.45	11.58	0.49	1.49
Experimental group	5.8	18.73	0.50	1.91
P value	0.129	< 0.001	0.951	< 0.001

[0097] Example 2. Effect of tryptophan-conjugated cholic acid on intestinal L cell line GLUTag

[0098] Materials: Mouse L cells (GLUTag cells from D. J. Drucker laboratory), cultured in high-glucose medium, and active GLP-1 detection kit (from Merck).

[0099] Methods: L cells were adherently cultured in high-glucose DMEM containing 10% fetal bovine serum to 80% cell density. Then, 50 mM tryptophan-conjugated cholic acid was diluted using the high-glucose medium to obtain tryptophan-conjugated cholic acid sample group with different final concentrations (0, 1, 10 or 50 μM). 1‰ DMSO solvent was used as a control. The different concentrations of tryptophan-conjugated cholic acid were added to mouse L cell samples, which were incubated for one hour, and the supernatant was isolated to detect the level of GLP-1 in the supernatant using an active GLP-1 detection kit (Merck).

[0100] Results: Compared with the control group, tryptophan-conjugated cholic acid increased the level of GLP-1 in the cell supernatant in a dose-dependent manner. The effect of promoting the GLP-1 level is obvious at a concentration of 10 μM (Table 3). The results are consistent with the in vivo data (Figure 5).

[0101] Table 3 Effect of tryptophan-conjugated cholic acid on GLP-1 secreted by intestinal L cell line GLUTag (The statistical method was a one-way analysis of variance with Tukey’s post-hoc test)

Group	Concentration of tryptophan-conjugated cholic acid (μM)			
	0	1	10	50

GLP-1 content (pM)	367.99	460.49	740.50	784.33
p value	Control group	0.206	< 0.001	< 0.001

[0102] Example 3. Detection of the yields of tryptophan-conjugated cholic acid produced by various probiotics

[0103] Materials: The strains used were shown in Table 4. All strains were routinely isolated without screening. Methods: When each strain was cultured (in MRS Culture medium, under 37°C anaerobic condition) to an OD value of 0.6, 1 mM bile acid and 1 mM tryptophan were added as substrates. After culture for additional 24 hours, 50ul of culture was taken and mixed with 50ul of methanol by shaking. The mixture was then centrifuged at 12000rpm, and the supernatant was filtered through a 0.22 μm pore size water-based filter membrane. Mass spectrometry was used to detect the content of tryptophan-conjugated cholic acid. The mass spectrometry conditions were as follows. A liquid chromatography-tandem mass spectrometry (LC-MS/MS) system was employed for the quantification of TrpCA. The LC-MS/MS system consists of an Acquity ultra-high performance liquid chromatography system (Waters Corporation, Milford, USA) coupled with a Sciex 5500 triple quadrupole linear ion trap mass spectrometer (AB Sciex, Framingham, MA, USA) in tandem. The chromatographic column used was ACQUITY UPLC CSH C18 (2.1 x 100 mm, 1.7 μm, Waters), with a column temperature of 40 °C and a flow rate of 0.25 mL/min. The injection volume was 5 μL. The mobile phase A was 0.1% formic acid aqueous solution, and the mobile phase B was 0.1% formic acid in acetonitrile. TrpCA was detected using the MRM mode under negative ion conditions. Gradient elution was used for the separation of metabolites: 0-1 min, 40% B; 1-6 min, 40-100% B; 6-7 min, 100% B; 7-8 min, 40% B. Mixed working standard solutions of different concentrations were obtained by gradient dilution (0.1, 1, 10, 100, 1000 nM) to construct a standard curve. CA-d4 (200 nM) was

used as an internal standard for content correction. The LC-MS/MS control system utilized the Analyst 1.6.2 version, and quantitative analysis was performed using the MultiQuant software (version 3.0.1).

[0104] Results: All the strains listed in Table 4 was able to synthesize tryptophan-conjugated cholic acid, and *Bifidobacterium animalis subsp. lactis* had the highest yield.

[0105] Table 4. Detection of tryptophan-conjugated cholic acid produced by various probiotics

Species	Genus	Content of TrpCA (nmol/L)
<i>Streptococcus salivarius subsp. thermophilus</i>	<i>Streptococcus</i>	24.4
<i>Pediococcus acidilactici</i>	<i>Pediococcus</i>	11.7
<i>Lactobacillus gasseri</i>	<i>Lactobacillus</i>	3.9
<i>Limosilactobacillus reuteri</i>	<i>Limosilactobacillus</i>	18.2
<i>Lactilactobacillus sakei</i>	<i>Lactilactobacillus</i>	18.6
<i>Lactobacillus acidophilus</i>	<i>Lactobacillus</i>	6.3
<i>Ligilactobacillus salivarius</i>	<i>Ligilactobacillus</i>	12.8
<i>Lactiplantibacillus plantarum</i>	<i>Lactiplantibacillus</i>	36.6
<i>Bifidobacterium animalis subsp. lactis</i>	<i>Bifidobacterium</i>	41
<i>Lactococcus lactis</i> (subspecies not determined)	<i>Lactococcus</i>	34.6

[0106] **Example 4. Effect of *Bifidobacterium animalis subsp. lactis* on indicators related to glucose tolerance in hyperglycemic mice**

[0107] Materials: The tested strain was *Bifidobacterium animalis subsp. lactis*. C57BL/6 mice were purchased from GemPharmatech, maintained at a temperature of 20-24°C, with a constant humidity of 50-60%, and exposed to light for 12 hours from 8:00 to 20:00 with soundproofing and with ad libitum feeding and drinking water. They were acclimated for one week before the experiment. The blood glucose meter was a product of Roche Company in Germany.

[0108] C57BL/6 mice were gavaged with the tested strains at a dose of 2×10^8 CFU per mouse once a week. The bacterial solution should be cultured in

advance, activated every week to ensure freshness, and the concentration was determined separately.

[0109] Methods: After 1 week of acclimation, 6-8 week-old male C57BL/6 mice (20-22g body weight) were evenly divided into groups based on their body weights: (1) control group, (2) test group: *Bifidobacterium animalis subsp. lactis* colonization group, with 7 mice in each group. Gavage was performed weekly for 2 consecutive weeks. The control group received an equal volume of saline. Two weeks later, a glucose tolerance test was conducted, and blood glucose levels were measured at different time points (0 min, 30 min, 60 min, 90 min, and 120 min) after glucose gavage. The area of the curve (AOC) was calculated. The GLP-1 and insulin secretion levels were detected using an active GLP-1 detection kit (from Merck) and an insulin detection kit (CUSABIO), respectively, according to the instructions provided in the kits.

[0110] Results: Colonization of *Bifidobacterium animalis subsp. lactis* could significantly improve the glucose tolerance and promote the secretion of GLP-1 and insulin. Fig. 6 shows the effect of *Bifidobacterium animalis subsp. lactis* on glucose tolerance of mice fed with a high-fat diet. The statistical method was two-way ANOVA on duplicate measurements with unpaired T test. * $P < 0.05$, ** $P < 0.01$. The upper panel of Fig. 6 shows the change in blood glucose level over time. The lower panel of Fig. 6 shows the AOC (area of the curve) of the control and treatment groups.

[0111] Table 5 Effects of colonization of *Bifidobacterium animalis subsp. lactis* on glucose-stimulated GLP-1 and insulin production in mice fed with a high-fat diet (The statistical method was a two-way analysis of variance of duplicate measurements with unpaired T test.)

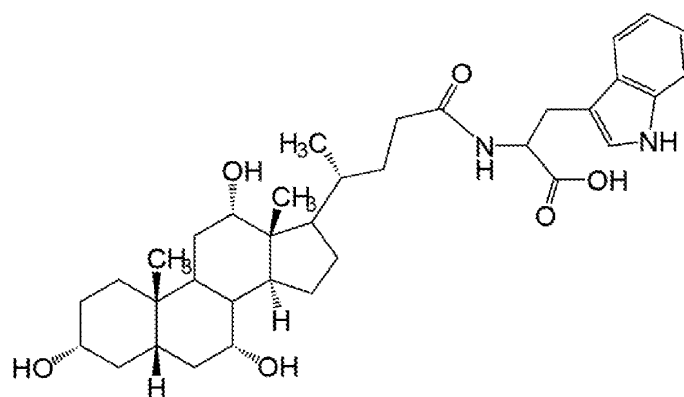
Group	GLP-1 (pM)		Insulin (ng/mL)	
	0 minute	15 min	0 minute	15 min
Control group	4.58	11.7	0.76	1.72

Test group	5.74	16.72	0.722	2.3
P value	0.185	< 0.001	0.969	0.002

[0112] Although the present disclosure has been described with reference to illustrative embodiments, it will be understood by those skilled in the art that various other changes, omissions and/or additions may be made and substantial equivalents may be substituted for elements of the described embodiments without departing from the spirit and scope of the present disclosure. In addition, many modifications may be made to adapt a particular situation or material to the teachings of the disclosure without departing from the scope thereof. Therefore, it is not intended to limit the disclosure to the particular embodiments disclosed for carrying out this disclosure, but that the disclosure will include all embodiments falling within the scope of the appended claims.

CLAIMS

1. Tryptophan-conjugated cholic acid (TrpCA) or a strain producing the same or a composition comprising the tryptophan-conjugated cholic acid or the strain producing the same for use in preventing and/or treating a metabolic disease or for use in promoting GLP-1 and/or insulin secretion, and/or for use in maintaining healthy blood glucose level, maintaining healthy blood lipid and/or regulating body fat; optionally wherein the tryptophan-conjugated cholic acid has the following structure:



;

optionally, wherein the composition is a pharmaceutical composition or a food composition.

2. The tryptophan-conjugated cholic acid or the strain producing the same or the composition comprising the tryptophan-conjugated cholic acid or the strain producing the same for use according to claim 1, wherein the strain belongs to one or more of the following genera: *Streptococcus*, *Pediococcus*, *Lactobacillus*, *Limosilactobacillus*, *Lactilactobacillus*, *Ligilactobacillus*, *Lactiplantibacillus*, *Bifidobacterium*, and *Lactococcus*.

3. The tryptophan-conjugated cholic acid or the strain producing the same or the composition comprising the tryptophan-conjugated cholic acid or the strain producing the same for use according to claim 1 or 2, wherein the strain belongs to one or more of the following species: *Streptococcus salivarius*, *Pediococcus acidilactici*, *Lactobacillus gasseri*, *Limosilactobacillus reuteri*, *Lactilactobacillus*

sakei, *Lactobacillus acidophilus*, *Ligilactobacillus salivarius*, *Lactiplantibacillus plantarum*, *Bifidobacterium animalis* and *Lactococcus lactis*.

4. The tryptophan-conjugated cholic acid or the strain producing the same or the composition comprising the tryptophan-conjugated cholic acid or the strain producing the same for use according to claim 3, wherein *Streptococcus salivarius* is *Streptococcus salivarius subsp. thermophilus*; and *Bifidobacterium animalis* is *Bifidobacterium animalis subsp. lactis*.

5. The tryptophan-conjugated cholic acid or the strain producing the same or the composition comprising the tryptophan-conjugated cholic acid or the strain producing the same for use according to any one of claims 1-4, wherein the metabolic disease is a GLP-1 related metabolic disease, optionally selected from diabetes or diabetes related diseases;

optionally, wherein the diabetes is selected from type 1 diabetes, type 2 diabetes, gestational diabetes, obesity diabetes, autoimmune diabetes and prediabetes;

optionally, wherein the disease associated with diabetes is selected from obesity, obesity-related disorders, metabolic syndrome, diabetic neuropathy, kidney diseases such as diabetic nephropathy, diabetic retinopathy, diabetic cardiomyopathy, cataract, macroangiopathy, osteopenia, hyperosmolar diabetic coma, diabetic gangrene, xerostomia, hypoacusis, diabetic cachexia, delayed wound healing, diabetic dyslipidemia, peripheral blood circulation disorders, non-alcoholic steatohepatitis, pre-diabetes, hyperlipemia, fatty liver disease, impaired fasting glucose, hyperglycemia, impaired glucose tolerance, insulin resistance, hyperinsulinemia, elevated blood fatty acids or glycerol levels, impaired wound healing, glucose intolerance, increased fasting glucose and dyslipidemia, insufficient numbers of pancreatic beta-cells, enteroendocrine cell insufficiency, glycosuria, metabolic acidosis, cataract, diabetic coronary heart disease, diabetic cerebrovascular diseases, diabetic peripheral vascular disease, atherosclerosis and stroke;

optionally, wherein the obesity is selected from symptomatic obesity, simple

obesity, childhood obesity, morbid obesity and abdominal obesity;

optionally, wherein the obesity related disease is selected from impaired glucose tolerance, abnormal lipid metabolism, hyperlipidemia, hypertension, heart failure, hyperuricemia, gout, fatty liver, coronary heart disease, cerebral infarction, sleep apnea syndrome, obesity hypoventilation syndrome and visceral obesity syndrome.

6. The tryptophan-conjugated cholic acid or the strain producing the same or the composition comprising the tryptophan-conjugated cholic acid or the strain producing the same for use according to any one of claims 1-5, wherein the food composition further comprises a food-acceptable carrier, diluent or excipient, and the pharmaceutical composition further comprises a pharmaceutically acceptable carrier, diluent or excipient;

optionally, wherein the food composition or pharmaceutical composition further comprises probiotics, and/or prebiotics;

optionally, wherein the composition is an oral composition, optionally selected from a group consisting of enteric coated tablets, granules, a capsule, tablets, powders, oral liquids, suspensions and emulsions;

optionally, wherein the composition is administrated at 1×10^{-1} to 10^{12} CFU/mL, 1×10^4 to 1×10^9 CFU/mL or 1×10^5 to 1×10^8 CFU/mL of at least one strain producing the tryptophan-conjugated cholic acid.

7. A composition for preventing and/or treating a metabolic disease, comprising tryptophan-conjugated cholic acid or a strain producing the same and further comprising another active ingredient for preventing and/or treating a metabolic disease, optionally, the another active ingredient is one or more of biguanides, sulfonylureas, glinides, alpha-glucosidase inhibitors, thiazolidinediones and GLP-1 drugs; optionally, the composition is an oral composition, optionally selected from the group consisting of enteric coated tablets, granules, capsules, tablets, powders, oral liquids, suspensions and emulsions;

optionally, wherein the composition is a food composition or a

pharmaceutical composition;

optionally, wherein the composition further comprises probiotics, prebiotics and/or pharmaceutically or food acceptable carriers, diluents or excipients;

optionally, wherein the composition is an oral composition, optionally selected from granules, capsules, tablets, powders, oral liquids, suspensions and emulsions.

8. A method for producing a tryptophan-conjugated cholic acid, comprising culturing probiotics in a medium in the presence of bile acid and tryptophan, wherein the probiotic is one or more of the following genera: *Streptococcus*, *Pediococcus*, *Lactobacillus*, *Limosilactobacillus*, *Lactilactobacillus*, *Ligilactobacillus*, *Lactiplantibacillus*, *Bifidobacterium*, and *Lactococcus*;

optionally, wherein the probiotics belongs to one or more of the following species: *Streptococcus salivarius*, *Pediococcus acidilactici*, *Lactobacillus gasseri*, *Limosilactobacillus reuteri*, *Lactilactobacillus sakei*, *Lactobacillus acidophilus*, *Ligilactobacillus salivarius*, *Lactiplantibacillus plantarum*, *Bifidobacterium animalis* and *Lactococcus lactis*;

optionally, wherein *Streptococcus salivarius* is *Streptococcus salivarius* subsp. *thermophilus*; and *Bifidobacterium animalis* is *Bifidobacterium animalis* subsp. *lactis*.

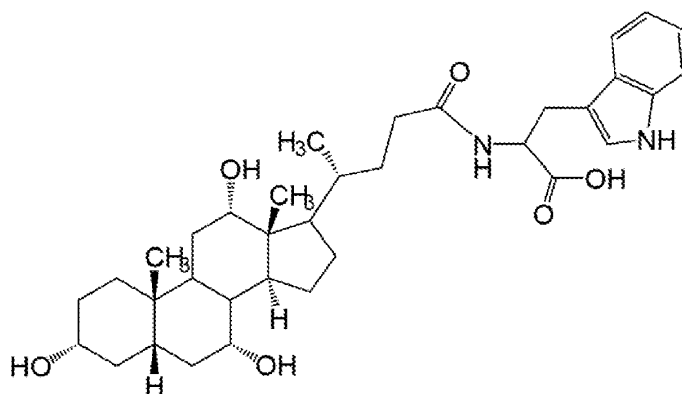
9. Use of probiotics for the production of a tryptophan-conjugated cholic acid, wherein the probiotic is one or more of the following genera: *Streptococcus*, *Pediococcus*, *Lactobacillus*, *Limosilactobacillus*, *Lactilactobacillus*, *Ligilactobacillus*, *Lactiplantibacillus*, *Bifidobacterium*, and *Lactococcus*;

optionally, wherein the probiotic is one or more of the following species: *Streptococcus salivarius*, *Pediococcus acidilactici*, *Lactobacillus gasseri*, *Limosilactobacillus reuteri*, *Lactilactobacillus sakei*, *Lactobacillus acidophilus*, *Ligilactobacillus salivarius*, *Lactiplantibacillus plantarum*, *Bifidobacterium animalis* and *Lactococcus lactis*;

optionally, wherein *Streptococcus salivarius* is *Streptococcus salivarius* subsp. *thermophilus*; and *Bifidobacterium animalis* is *Bifidobacterium animalis*

subsp. lactis;

optionally, wherein the tryptophan-conjugated cholic acid has the following structure:



10. A method for screening probiotics that promote GLP-1 and/or insulin secretion or production, comprising:

- (1) culturing probiotics in the presence of bile acids and tryptophan to obtain culture supernatant;
- (2) measuring the content of the tryptophan-conjugated cholic acid in the culture supernatant; and
- (3) selecting the probiotics which produce the tryptophan-conjugated cholic acid;

optionally, wherein the probiotics belongs to one or more of the following genera: *Streptococcus*, *Pediococcus*, *Lactobacillus*, *Limosilactobacillus*, *Lactilactobacillus*, *Ligilactobacillus*, *Lactiplantibacillus*, *Bifidobacterium*, and *Lactococcus*;

optionally, wherein the probiotics belongs to a species selected from one or more of *Streptococcus salivarius*, *Pediococcus acidilactici*, *Lactobacillus gasseri*, *Limosilactobacillus reuteri*, *Lactilactobacillus sakei*, *Lactobacillus acidophilus*, *Ligilactobacillus salivarius*, *Lactiplantibacillus plantarum*, *Bifidobacterium animalis* and *Lactococcus lactis*;

optionally, wherein *Streptococcus salivarius* is *Streptococcus salivarius*

subsp. thermophilus; and *Bifidobacterium animalis* is *Bifidobacterium animalis subsp.lactis*;

optionally, wherein the method comprises mixing the culture medium of the probiotics with methanol and detecting the content of the tryptophan-conjugated cholic acid by mass spectrometry;

optionally, wherein the tryptophan-conjugated cholic acid has the following structure:

