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(54) **Title:** OBESITY ANIMAL MODEL AND METHODS FOR MAKING AND USING THEREOF

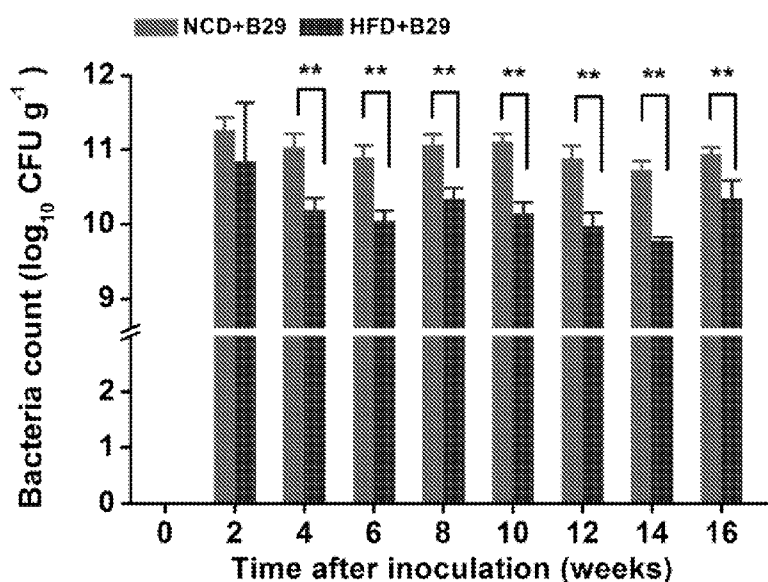


FIGURE 1

(57) **Abstract:** The application provides, among others, methods for constructing animal obesity models, methods for screening microorganism or composition of microorganisms that may cause obesity, methods for screening therapeutic targets for treating metabolic disorders, and methods for screening or evaluating microorganisms, compounds, food, recipes, formulations, drugs, nutritional supplements, health-care products, beverages and other items for preventing and treating metabolic diseases.

SUMMARY

The following summary is illustrative only and is not intended to be in any way limiting. In addition to the illustrative aspects, embodiments, and features described above, further aspects, embodiments, and features will become apparent by reference to the drawings and the following detailed description.

In one aspect, the present application relates to methods for constructing animal obesity model. In one embodiment, the method includes inoculating animals with an endotoxin (LPS)-producing bacterium under the high-fat diet condition to produce a gnotobiotic animal obesity model. After a microorganism, such as an endotoxin-producing bacterium, or a composition including an endotoxin-producing bacterium, is inoculated into germ-free animals fed with a high-fat diet, the microorganism colonizes within the guts of the germ-free animals. The animals may display obesity, insulin resistance, chronic inflammation, or other symptoms of metabolic disorder, immune system disorder, or other systemic disorders.

In another aspect, the application provides methods for screening microorganism or composition of microorganisms that may cause obesity.

In another aspect, the application provides methods for screening therapeutic targets for treating metabolic disorders such as obesity.

In another aspect, the application provides methods for screening or evaluating microorganisms, food, recipes, formulations, compositions, compounds, drugs, nutritional supplements, healthcare products, beverages, or other items that can be used for preventing or treating metabolic diseases such as obesity.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other features of this disclosure will become more fully apparent from the following description and appended claims, taken in conjunction with the accompanying drawings. Understanding that these drawings depict only several embodiments arranged in accordance with the disclosure and are, therefore, not to be considered limiting of its scope, the disclosure will be described with additional specificity and detail through use of the accompanying drawings, in which:

FIGURE 1 illustrates the colonization of B29 isolates in gut of germ-free mice fed with a high-fat diet by comparing the population levels of *Enterobacter* sp. B29 in the *Enterobacter*-associated gnotobiotic model of obesity mice with those of the control mice:

FIGURE 2 illustrates B29's effects on obesity and insulin resistance symptoms of germ-free mice fed with a high-fat diet; FIGURE 2A shows the body weight of the *Enterobacter*-associated gnotobiotic model of obesity and the controls; FIGURE 2B shows mass of epididymal, mesenteric, subcutaneous inguinal and retroperitoneal fat pad of the *Enterobacter*-associated gnotobiotic model of obesity and the controls; FIGURE 2C shows abdominal photographs of the *Enterobacter*-associated gnotobiotic model of obesity and the controls; FIGURE 2D shows oral glucose tolerance test (OGTT) and area under the curve (AUC) of the *Enterobacter*-associated gnotobiotic model of obesity mice and the controls; and FIGURE 2E shows serum 2 h-post load insulin levels of the *Enterobacter*-associated gnotobiotic model of obesity and the controls; and

FIGURE 3 illustrates B29's effect on level of inflammation of germ-free mice fed with a high-fat diet; FIGURE 3A shows serum LBP levels of the *Enterobacter*-associated gnotobiotic model of obesity and the controls; FIGURE 3B shows serum SAA levels of the *Enterobacter*-associated gnotobiotic model of obesity and the controls; FIGURE 3C shows serum adiponectin corrected for bodyweight levels of the *Enterobacter*-associated gnotobiotic model of obesity and the controls; FIGURE 3D shows reverse transcription (RT)-quantitative PCR analysis of expression of *Tnfa*, *Il1 β* , *Il6*, *Mcp1*, *Ikk ϵ* and *Tlr4* in the liver of the *Enterobacter*-associated gnotobiotic model of obesity and the controls; FIGURE 3E shows reverse transcription (RT)-quantitative PCR analysis of expression of *Tnfa*, *Il1 β* , *Il6*, *Mcp1*, *Ikk ϵ* and *Tlr4* in the epididymal fat pad of the *Enterobacter*-associated gnotobiotic model of obesity and the controls; and FIGURE 3F shows reverse transcription (RT)-quantitative PCR analysis of expression of *Tnfa*, *Il1 β* , *Il6*, *Mcp1*, *Ikk ϵ* and *Tlr4* in the ileum of the *Enterobacter*-associated gnotobiotic model of obesity and the controls.

DETAILED DESCRIPTION

In the following detailed description, reference is made to the accompanying drawings, which form a part hereof. In the drawings, similar symbols typically identify similar components, unless context dictates otherwise. The illustrative embodiments described in the detailed description, drawings, and claims are not meant to be limiting. Other embodiments may be utilized, and other changes may be made, without departing from the spirit or scope of the subject matter presented herein. It will be readily understood that the aspects of the present disclosure, as generally described herein, and illustrated in the Figures, can be arranged, substituted, combined,

separated, and designed in a wide variety of different configurations, all of which are explicitly contemplated herein.

The application generally provides, among others, novel methods for constructing animal obesity models, novel methods for screening microorganism or composition of microorganisms that may cause obesity, novel methods for screening therapeutic targets for treating metabolic disorders and novel methods for screening or evaluating microorganisms, compounds, compositions, food, recipes, formulations, drugs, nutritional supplements, healthcare products, beverages, and other items for preventing and treating metabolic diseases.

In one example, the present application provides a method for providing an animal obesity model, in which germ-free animals are inoculated with a microorganism while fed with a high-fat diet. The microorganism may be a single microorganism or a combination of microorganisms. The high fat diet may contain at least 5% fat. The microorganism or the combination may colonize within the guts of the germ-free animals and form the basis of gut microbiota population for the resulting animal models. The animal model may exhibit the symptoms of at least one metabolic or immune system disorders.

The symptoms of metabolic or immune system disorders may be any metabolic syndrome or immune system disorders including, without limitation, obesity, insulin resistance, chronicle inflammation, or non-alcoholic fatty liver disease.

The germ-free animal may be mammals or non-mammals. Example mammals may include a mouse, a rat, a Guinea pig, a pig, a rabbit, or a monkey.

The fat content of the high-fat diet may be at least 5%, at least 10%, at least 15%, at least 20%, or at least 25%.

The microorganism may be a bacterium such as an endotoxin-producing bacterium. The endotoxin-producing bacterium refers to a bacterium capable of producing substance(s) that has endotoxin activity. Example endotoxin-producing bacterium may include, without limitation, B29 strain of *Enterobacter cloacae*, *Enterobacter cloacae*, *Enterobacter*, enterobacteriaceae, γ -*Proteobacteria*, *Proteobacteria*, or Gram-negative bacteria. The microorganism may be in a composition including multiple strains of endotoxin-producing bacteria or including strains of endotoxin-producing and non-endotoxin-producing bacteria. In one example, the microorganism may contain an endotoxin synthesis gene whose sequence is at least 15%, 20%, 30%, 50%, 75% or 95% similar to the B29 strain of *Enterobacter cloacae*.

The animal obesity model provided above may include a gut bacterium having a 16S rRNA gene whose sequence is at least 15%, 20%, 30%, 50%, 75% or 95% similar to SEQ ID NO.1.

5 The inoculation method as noted above may be any inoculation or administration methods in biological, medicinal, or pharmaceutical fields. Example inoculation methods may include gavage, diets addition, drinking water addition, and smear or topical application of skin or fur. After inoculation, the inoculated microorganisms such as bacteria may colonize in the animal gut, or may be detected in animal feces. In one example, the inoculated bacterial may be continuously detected in the animal feces. The animals may exhibit, among other related
10 metabolic syndromes, weight gain, increased fat, blood lipids, insulin resistance, serum lipopolysaccharide binding protein or leptin resistance, increased serum level of inflammation, or increased inflammatory factor expression levels.

In another aspect, the present application provides methods for screening an obesity-causing microorganism or a combination thereof. In one example, a positive control bacterium
15 that is capable of colonizing the gut of a germ-free animal is inoculated to the animal, which is fed with a high-fat diet. The resulting animal may exhibit at least one type of metabolic or immune system disorders. The said positive control bacterium is compared with a test microorganism or combination thereof. If the test microorganism or a combination thereof exhibits similar effect as the positive control, it is indicative that the test microorganism or
20 combination thereof may cause obesity.

In another aspect, the present application provides methods for screening a microorganism or a combination thereof that has a preventive or therapeutic effect on the symptom of metabolic or immune system disorder. In one example, the method uses an animal model that exhibits at least one symptom of metabolic or immune system disorders. After
25 administering a test microorganism or a combination thereof, if the animal exhibits improvement in at least one symptom of metabolic or immune system disorders, it is indicative that the said microorganism or a combination thereof has a preventive or therapeutic effect on the symptom of metabolic or immune system disorders. In one example, the improvement in at least one symptom of metabolic or immune system disorders may include amelioration of symptom of
30 obesity, insulin resistance, or chronic inflammation. For example, after administering a test microorganism or a combination thereof, if the animal exhibits one or more of weight loss, reduced fat, decreased blood lipids, improved insulin resistance, reduced level of serum

lipopolysaccharide binding protein, decreased leptin resistance, decreased serum level of inflammation, or decreased inflammatory factor expression levels, it is indicative that the test microorganism or the combination thereof may have a preventive or therapeutic effect on the symptom of metabolic or immune system disorder.

5 The test microorganism may be any microorganism. In one example, the test microorganism may include lactic acid bacteria, *Bifidobacteria*, butyrate-producing bacteria, Gram-positive cocci or probiotics.

 In another aspect, the present application provides methods for screening a material that may have a preventive or therapeutic effect on the symptom of metabolic or immune system disorders. In one example, the method uses an animal model exhibiting at one symptom of metabolic or immune system disorders. After administering the material, if the animal exhibits improvement on at least one symptom of metabolic or immune system disorders, it is indicative of that the material may have a preventive or therapeutic effect on the symptom of metabolic or immune system disorders. For example, the improvement may include weight loss, decreased insulin resistance, or reduced chronic inflammation. In one example, after administering the material, if the gnotobiotic animal obesity model exhibits one or more of weight loss, reduced fat, decreased blood lipids, improved insulin resistance, reduced level of serum lipopolysaccharide binding protein, decreased leptin resistance, decreased serum level of inflammation, or decreased inflammatory factor expression levels, it is indicative that the material may have a preventive or therapeutic effect on the symptom of metabolic or immune system disorder.

 The above noted material may be edible. For example, the material may include, without limitation, food, recipes, formulations, compounds, compositions, drugs, nutritional supplement, healthcare product, or beverage.

 The following examples are for illustration of the execution and property of representative method of the application. These examples are not intended to limit the scope of the application.

EXAMPLE 1

Materials and Animal Models

 Male C57BL/6J mice (germ-free) were used as an example animal. *Enterobacter cloacae* B29 were used as an example inoculation microorganism. The said *Enterobacter cloacae* B29 refers to a superior strain isolated from feces of a morbidly obese patient volunteer. The 16s RNA gene sequence analysis and biochemical assays identified the bacteria as an *Enterobacter cloacae*.

24 healthy male C57BL/6J mice (germ-free) were purchased from Research Diets, Inc. (New Brunswick, NJ) and fed either on a normal chow diet (NCD, fat content 4.62%, 3.45 kcalories/gram) or high-fat diet (HFD, fat content 34.9%, 5.21 kcalories/gram)

Method on mice: mice were maintained in a 12-hour light cycle (light starting from 6:30 AM) at 22±3 °C. Six-ten weeks old sterile mice were randomly distributed into four groups (6 per group), and received the following treatment: (1), NCD+LB group: this group was inoculated by gavage with 0.1ml sterile LB medium, and fed a NCD; (2) NCD+B29 group: the group was inoculated by gavage with B29 (10^2 cells) suspension in 0.1ml sterile LB medium and fed a NCD; (3) HFD+LB group: this group was inoculated by gavage with 0.1ml sterile LB medium and fed a HFD; and (4) HFD+B29 group: this group was inoculated by gavage with B29 (10^2 cells) suspension in 0.1ml sterile LB medium and fed a HFD. The experiment lasted 16 weeks. Mice were raised in cages of 3 or 4 in the gnotobiotic isolators, and each mouse's body weight was measured every week. Each group of animals was housed in their own isolators fed with sterile diet and sterile water until euthanasia, and surveillance for bacterial contamination was performed by a periodic bacteriologic examination of the gnotobiotic isolators.

During the experiment, the body weight, fat pad weight, insulin sensitivity and inflammation levels of the mice were monitored.

EXAMPLE 2

Isolation and Characterization of *Enterobacter cloacae* B29

An analysis of the obese population revealed that a majority of morbidly obese patients suffers from gross imbalance in the gut microbiota. For example, 35% of the total gut bacteria were found to be *Enterobacter* (assayed through 16S rRNA gene library) in a morbidly obese volunteer (26 year old, male, Han ethnicity, body weight 174.9 kg, BMI 58.78 kg/m²). Meanwhile, physical exams showed that the volunteer suffers severe metabolic syndrome. The volunteer underwent 23-weeks of dietary intervention (composition: whole grains, traditional Chinese medicine and prebiotics), when he lost 30.1 kg after 9 weeks, 51.4 kg of 174.8 kg after 23 weeks, and exhibited improvement in all measurement of metabolic syndrome. An analysis of the gut bacteria population revealed that the *Enterobacter* population reduced to 1.8% after 9 weeks on the diet, and became undetectable at the end of the 23-week trial. *Enterobacter* strains were isolated from the feces of the volunteer via a "sequence-guided-isolation" scheme using LB medium (Luria-Bertani, recipe attached below) at 37°C, obtained the most abundant isolates, and named it B29. Full-length sequencing analysis were performed on 16S rRNA of

B29, and it was found that B29 is 99% homologous to *Enterobacter cloacae* (GenBank accession no. AB244457). A biochemical test with VITEK 2 Gram-negative card GN card, bioMérieux, Marcy l'Étoile, France) also indicated that B29 is 98% likely to belong to *Enterobacter cloacae* (Table 1).

5 Lipopolysaccharide (LPS) were isolated from B29 using a LPS isolation kit from iNtRON Biotechnology Co., Seoul, Korea, and endotoxin activity level was measured using an endotoxin activity assay kit LAL (ACC, USA). The result showed that the endotoxin activity of B29 is 4.45×10^6 EU mg⁻¹ LPS, comparable to *E. coli* strain 055:B5.

10 In order to perform *Enterobacter cloacae* B29-specific detection, a strain B29 that is resistant to 500 ug/ml Rifampicin was isolated using Rifampicin resistant strains screening method. This strain was inoculated into LB medium and shook for 12 hours under 37°C, and the resulting bacteria culture was used to construct the animal model.

15 The formula for LB medium (Luria-Bertani) was as follows: tryptone 10g/L, yeast extract 5g/L, NaCl 10g/L, Ph7.4. LB solid medium: 15 grams of agarose were added into 1L liquid LB medium, heat dissolved, and the medium were poured into plate before cooling.

TABLE 1. Biochemical Characterization of *Enterobacter cloacae* B29 with VITEK 2 ID-GN system

Well	Abbreviation	Assay	result
2	APPA	Ala-Phe-Pro-Arylamidase	-
3	ADO	Adonitol	+
4	PyrA	L-Pyrrolydonyl-Arylamidase	-
5	lARL	L- Arabitol	-
7	dCEL	D- Cellobiose	+
9	BGAL	β- d-Galactosidase	+

10	H2S	H2S production	-
11	BNAG	β -N-acetyl-glucosaminidase	+
12	AGLTp	Glutamyl Arylamidase pNA	-
13	dGLU	D-Glucose	+
14	GGT	γ - Glutamyltransferase	-
15	OFF	Glucose fermentation	+
17	BGLU	β - Glucosidase	(-)
18	dMAL	D- Maltose	+
19	dMAN	D- Mannitol	+
20	dMNE	D- Mannose	+
21	BXYL	β - Xylosidase	+
22	BAlap	β - Alanine arylamidase pNA	-
23	ProA	L- Proline Arylamidase	+
26	LIP	Lipase	-
27	PLE	Palatinose	+
29	TyrA	Tyrosine Arylamidase	+

31	URE	Urease	-
32	dSOR	D- Sorbitol	+
33	SAC	Saccharose/Sucrose	+
34	dTAG	D- Tagatose	-
35	dTRE	D- Trehalose	+
36	CIT	Citric acid salt (sodium)	+
37	MNT	Malonic acid salts	+
39	5KG	5-Keto-glucoside	-
40	ILATk	L-Lactate alkalinisation	+
41	AGLU	α -Glucose	(-)
42	SUCT	Succinate alkalinisation	+
43	NAGA	N-Acetyl- β -d-Galactose ammonia-lyase	+
44	AGAL	α - Galactose glucoside enzyme	+
45	PHOS	Phosphatase	-
46	GlyA	Glycine Arylamidase	+
47	ODC	ornithine decarboxylase	+

48	LDC	Lysine decarboxylase	-
53	IHISa	Histidine assimilation	-
56	CMT	COURMARATE	-
57	BGUR	β - Glucuronidase	-
58	O129R	O/129 tolerance	+
59	GGAA	Glu-Gly-Arg- Arylamidase	-
61	IMLTa	L- Malate assimilation	-
62	ELLM	ELLMAN	-
64	ILATa	L- Lactate assimilation	-

EXAMPLE 3

B29 Stable Colonization in the Gut of Germ-free Mouse

During the experiment, fresh feces samples were collected every two weeks, weighed
5 immediately, and diluted 1:10 in sterile 0.01M PBS. PBS buffer was made according to the
following formula: 135 mM NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, and 8 mM K₂HPO₄ , pH 7.2.
Take 100 μ l suspension and spread them on LB plates, cultured at 37°C for 16 hours before
counting colonies. The results showed that, during the experiment period (0-16 weeks), the
model mice (i.e., HDF+B29) and the NCD+B29 mice all produced feces that contained
10 *Enterobacter cloacae* B29 at the density of 10¹⁰-10¹² bacteria per gram of wet feces. This result
showed that *Enterobacter cloacae* B29 can stably colonize in the gut of germ-free mice after only
one inoculation (FIGURE 1).

EXAMPLE 4

B29 Strain Induces Obesity and Insulin Resistance in Mice Fed a High Fat Diet

During the experiment, the body weight of each mouse was measured accurately with an electronic balance ($d = 0.01$) once a week. On the week 16, mouse was orally administered glucose (2 g/kg body weight) after 5 hours' fasting. Immediately before administering glucose (0 minutes), and 15, 30, 60 and 120 minutes after administering glucose, blood was collected from tail vein, and blood glucose levels were immediately measured by Roche blood glucose meter. These data points were used to calculate the blood glucose tolerance test. 120 minutes after administering glucose, 50 μ l blood were collected from retro-orbital vein, left in room temperature for one hour; serum were collected by centrifuge at 3000 rpm for 15 minutes; and blood insulin levels were measured by ELISA (Mercodia, Uppsala, Sweden). This data point represents insulin level two hours after diet. At the end of week 16, mice were dissected under the following procedures. First, open abdomen pictures were taken with rulers as marker on two representative mice from each group; subsequently, epididymal fat pads, mesenteric fat pads, groin subcutaneous fat pads and perirenal fat pads from mice of each group were collected and weighed accurately. Statistical analyses were performed and the results were expressed at average $\pm$ S.E.M, using single factor analysis of variance (post Hoc) for statistical significance analysis (Turkey's multiple comparison test, SPSS 17.0). The results are shown in FIGURE 2.

The results showed that, the model mice that fed a high-fat diet and received inoculation of *Enterobacter cloacae* B29 exhibited significantly higher body weight than the other three control groups. At the end of 16 weeks, the model mice demonstrated very obvious obesity phenotype. In addition, the results from epididymal fat pads, mesenteric fat pads, groin subcutaneous fat pads and perirenal fat pads demonstrated that the model mice receiving a high-fat diet and inoculation of *Enterobacter cloacae* B29 had very significant weight increases in major fat pads of the mice body. Abdominal dissection pictures demonstrated that the model mice group's body mass and abdominal fat accumulations are obviously larger than those of other groups. Finally, the glucose tolerance of the model group mice is markedly significantly lower than that of other groups, whereas the insulin levels two-hours after diet is significantly higher in the model group compared to other control group.

EXAMPLE 4

Enterobacter cloacae B29's Effects on the Level of Inflammation of Mice Fed a High-Fat Diet

In order to demonstrate *Enterobacter cloacae* B29's effects on the level of inflammation in mice fed a high-fat diet, the concentrations of serum lipopolysaccharide binding protein (LBP), serum amyloid A (SAA) protein and adiponectin were measured. The gene expression levels of

inflammatory factors in liver, epididymal fat pad, and jejunal tissue were also quantitatively determined. The results are shown in FIGURE 3.

The LBP level in obesity model mice (HFD+B29) was significantly higher than that of other groups, despite the numbers of B29 in the gut of mice fed a normal diet far exceeded all other groups. Because B29 was the only LPS-producing bacterium, the significant increase in the endotoxin level in the model mice sera can only derive from B29. The significant increase in the endotoxin level in serum will result in systemic inflammation, which in turn led to insulin resistance and other metabolic disorders symptoms. The obesity model mice showed significant increase in SAA level and significant decrease in adiponectin level, confirming the significant increase in systemic inflammation. The result of gene expression levels of inflammatory factors in liver, epididymal fat pad, and jejunal tissue showed that the obesity model mice had the highest level of expression of *TNF α* , *IL-1 β* , *IL-6*, *IKK- ϵ* , and *TLR4*.

Thus, in the obesity model mice that is created based on this application, the serum LBP level and serum SAA level increased significantly compared to the three control groups, adiponectin level decreased significantly compared to the three control groups, and the local tissue expression levels of pro-inflammatory genes increased significantly, compared to the three control groups.

As used in this document, the singular forms “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Nothing in this disclosure is to be construed as an admission that the embodiments described in this disclosure are not entitled to antedate such disclosure by virtue of prior invention. As used in this document, the term “comprising” means “including, but not limited to.”

In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

As will be understood by one skilled in the art, for any and all purposes, such as in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be

readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as “up to,” “at least” and the like include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each
5 individual member. Thus, for example, a group having 1-3 cells refers to groups having 1, 2, or 3 cells. Similarly, a group having 1-5 cells refers to groups having 1, 2, 3, 4, or 5 cells, and so forth.

From the foregoing, it will be appreciated that various embodiments of the present disclosure have been described herein for purposes of illustration, and that various modifications may be made without departing from the scope and spirit of the present disclosure. Accordingly,
10 the various embodiments disclosed herein are not intended to be limiting, with the true scope and spirit being indicated by the following claims.

WHAT IS CLAIMED IS:

1. A non-human obesity animal model whose gut microbiota population comprises at least one bacterium having a 16S rRNA gene, wherein the sequence of the 16S rRNA gene is at least 75% similar to SEQ ID NO.1.
2. The non-human obesity animal model of Claim 1, wherein the bacterium is an endotoxin-producing bacterium.
3. The non-human obesity animal model of Claim 1, wherein the bacterium is *Enterobacter*.
4. The non-human obesity animal model of Claim 1, wherein the bacterium is *Enterobacter cloacae*.
5. The non-human obesity animal model of Claim 1, wherein the 16S rRNA gene is at least 95% similar to SEQ ID NO.1.
6. The non-human obesity animal model of Claim 1, wherein the 16S rRNA gene is at least 85% similar to SEQ ID NO.1.
7. The non-human obesity animal model of Claim 1, wherein the non-human obesity animal model is a mouse, a rat, a Guinea pig, a pig, a rabbit, or a Monkey.
8. A method for establishing an obesity animal model, comprising,

inoculating a germ-free animal with a bacterium having a 16S rRNA gene to provide an inoculated animal, wherein the sequence of the 16S rRNA gene is at least 75% similar to SEQ ID NO.1; and

feeding the inoculated animal with a diet having at least 5% of fat content for a period of time to provide an obesity animal model.

9. The method of Claim 8, wherein the obesity animal model has a gut microbiota population comprising at least one bacterium having a 16S rRNA gene, wherein the sequence of the 16S rRNA gene is at least 75% similar to SEQ ID NO.1.
10. The method of Claim 8, wherein the obesity animal model manifests a metabolic syndrome.
11. The method of Claim 10, wherein the metabolic syndrome comprises obesity, insulin resistance, chronic inflammation, or a combination thereof.
12. The method of Claim 8, wherein the bacterium is an endotoxin-producing bacterium.
13. The method of Claim 8, wherein the bacterium is *Enterobacter*.
14. The method of Claim 8, wherein the bacterium is *Enterobacter cloacae*.
15. The method of Claim 8, wherein the sequence of the 16S rRNA gene is at least 95% similar to SEQ ID NO.1.
16. The method of Claim 8, wherein the non-human obesity animal model is a mouse, a rat, a rabbit, a monkey, a pig, or a Guinean pig.
17. The method of Claim 8, wherein the period of time is at least 4 weeks.
18. The method of Claim 8, wherein the inoculating the germ-free animal comprises intragastric administration, topical administration, oral administration, or a combination thereof.
19. The method of Claim 8, wherein the inoculating the germ-free animal comprises inoculating the germ-free animal with at least 10^2 bacteria cells.
20. The method of Claim 8, wherein the diet has at least 20% of fat content.
21. The method of Claim 8, wherein the diet has at least 10% of fat content.
22. The method of Claim 8, wherein the diet comprises at least 4.5 kcal per gram.

23. A method for establishing an obesity animal model, comprising,
- inoculating a germ-free animal with an endotoxin-producing bacterium to provide an inoculated animal; and
- feeding the inoculated animal with a diet having at least 5% of fat content for a period of time to provide an obesity animal model, wherein the obesity animal model manifests at least one metabolic syndrome.
24. The method of Claim 23, wherein the endotoxin-producing bacterium comprises a bacterium of *Enterobacter cloacae*.
25. The method of Claim 23, wherein the endotoxin-producing bacterium comprises a bacterium of *Enterobacter*.
26. The method of Claim 23, wherein the endotoxin-producing bacterium comprises a bacterium of *Enterobacteriaceae*.
27. The method of Claim 23, wherein the endotoxin-producing bacterium comprises a bacterium of γ -*Proteobacteria*.
28. The method of Claim 23, wherein the endotoxin-producing bacterium comprises a bacterium of *Proteobacteria*.
29. The method of Claim 23, wherein the endotoxin-producing bacterium comprises a bacterium of Gram-negative bacteria.
30. The method of Claim 23, wherein the endotoxin-producing bacterium is capable of colonizing in the germ-free animal's gut.
31. The method of Claim 23, wherein the endotoxin-producing bacterium has a 16S rRNA gene, and wherein the sequence of the 16S rRNA gene is at least 75% similar to SEQ ID NO.1.

32. The method of Claim 23, wherein the obesity animal model has a gut microbiota population comprising at least one bacterium having a 16S rRNA gene, wherein the sequence of the 16S rRNA gene is at least 75% similar to SEQ ID NO.1.
33. A method for screening a compound, comprising,
- providing a test obesity animal model and a control obesity animal model, wherein both the test obesity animal model and the control obesity animal model have a gut microbiota population comprising at least one bacterium having a 16S rRNA gene, and wherein the sequence of the 16S rRNA gene is at least 75% similar to SEQ ID NO.1;
- providing a compound to the test obesity animal model for a period of time while feeding a diet having at least 5% of fat content to both the test obesity animal model and the control obesity animal model over the period of time; and
- measuring weight gain, serum level change of an endotoxin biomarker, or both in the test obesity animal model and in the control obesity animal model, and wherein the weight gain of the test obesity animal model being statistically significantly less than the weight gain of the control obesity animal model, or the serum level change of the endotoxin biomarker in the test obesity animal model being statistically insignificant while the serum level change of endotoxin biomarker in the control obesity animal model being statistically significant, or both is indicative that the compound is effective in preventing metabolic syndrome.
34. The method of Claim 33, wherein the compound is a food or a drug.
35. The method of Claim 33, wherein the endotoxin biomarker comprises lipopolysaccharide-binding protein (LBP), lipopolysaccharide (LPS), or a combination thereof.
36. The method of Claim 33, wherein the metabolic syndrome comprises weight gain, obesity, insulin resistance, chronic inflammation, or a combination thereof.
37. A method for screening an obesity preventing bacterium, comprising,

providing a test obesity animal model and a control obesity animal model, wherein both the test obesity animal model and the control obesity animal model have a gut microbiota population comprising at least one bacterium having a 16S rRNA gene, and wherein the sequence of the 16S rRNA gene is at least 75% similar to SEQ ID NO.1;

inoculating the test obesity animal model with a test bacterium to provide an inoculated test obesity animal model;

feeding a diet having at least 5% of fat content to both the inoculated test obesity animal model and the control obesity animal model for a period of time; and

measuring weight gain, serum level change of an endotoxin biomarker, or both in the inoculated test obesity animal model and in the control obesity animal model, wherein the weight gain of the inoculated test obesity animal model being statistically significantly less than the weight gain of the control obesity animal model, or the serum level change of the endotoxin biomarker in the inoculated test obesity animal model being statistically insignificant while the serum level change of endotoxin biomarker in the control non-human obesity animal model being statistically significant, or both is indicative that the test bacterium is effective in preventing metabolic syndrome.

38. The method of Claim 37, wherein the endotoxin biomarker comprises lipopolysaccharide-binding protein (LBP), lipopolysaccharide (LPS), or a combination thereof.
39. The method of Claim 37, wherein the bacterium comprises an isolate of gut bacterium of a subject.
40. The method of Claim 37, wherein the subject is a human.
41. The method of Claim 37, wherein the metabolic syndrome comprises weight gain, obesity, insulin resistance, chronic inflammation, or a combination thereof.
42. A method for screening an obesity causing bacterium, comprising,

inoculating a germ-free animal with a test bacterium to provide a test animal;

feeding a diet having at least 5% of fat content to both the test animal and a control obesity animal model for a period of time, wherein the control obesity animal model has a gut microbiota population comprising at least one bacterium having a 16S rRNA gene, and wherein the sequence of the 16S rRNA gene is at least 75% similar to SEQ ID NO.1; and

measuring weight gain, serum level change of an endotoxin biomarker, or both in the test animal and in the control obesity animal model, wherein the weight gain of the test animal model being at least statistically similar to the weight gain of the control obesity animal model, or the serum level change of the endotoxin biomarker in the test animal being at least statistically similar to the serum level change of the endotoxin biomarker in the control obesity animal model, or both is indicative that the test bacterium increases the incidence of metabolic syndrome.

43. The method of Claim 42, wherein the endotoxin biomarker comprises lipopolysaccharide-binding protein (LBP), lipopolysaccharide (LPS), or a combination thereof.
44. The method of Claim 42, wherein the metabolic syndrome comprises weight gain, obesity, insulin resistance, chronic inflammation, or a combination thereof.

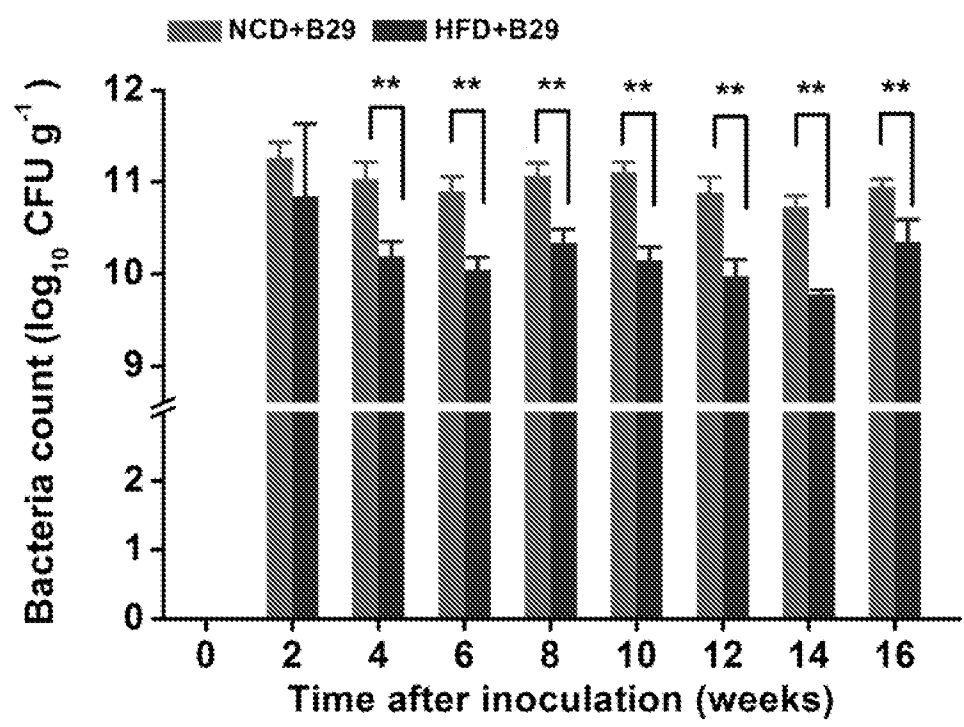


FIGURE 1

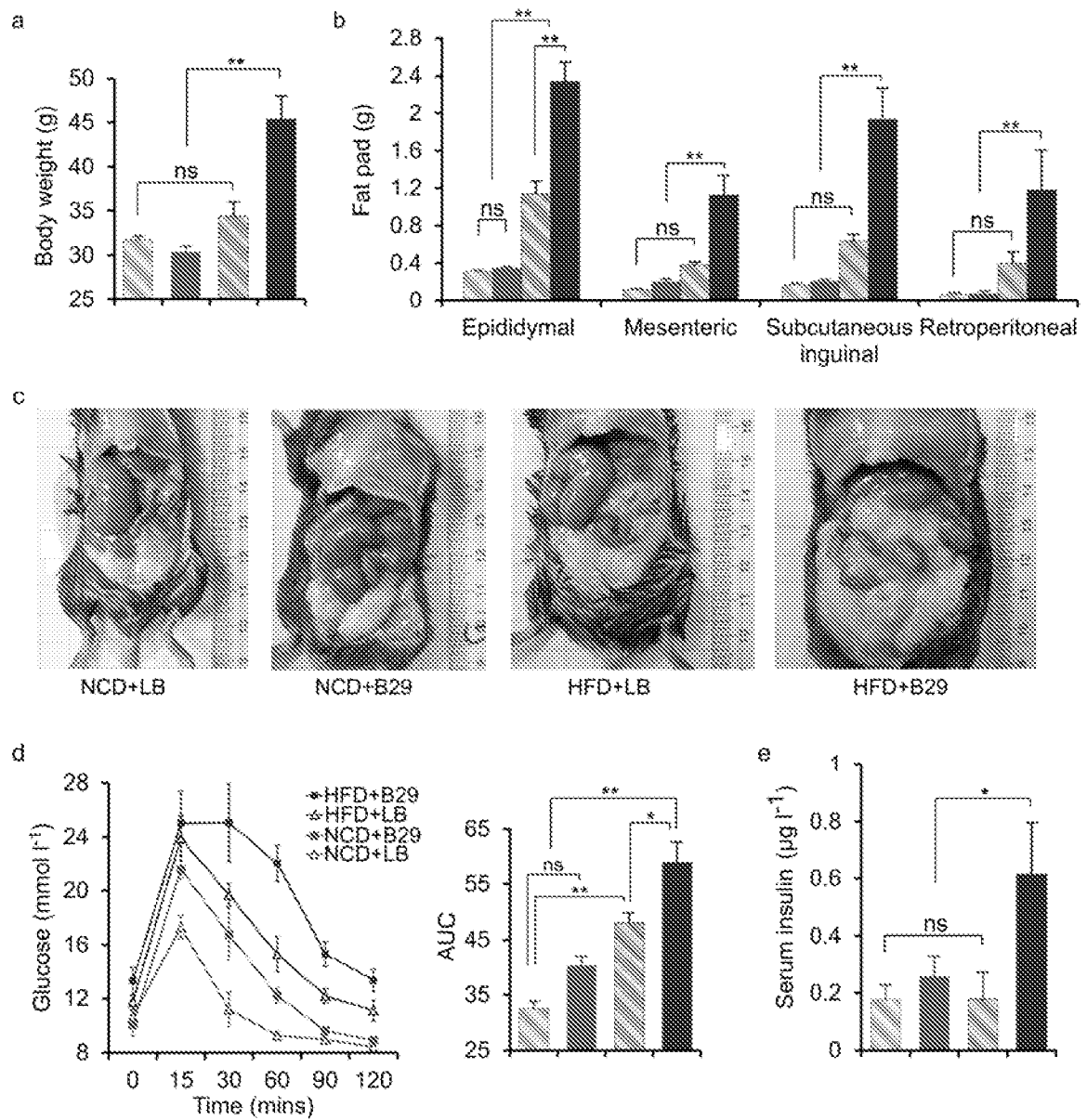


FIGURE 2

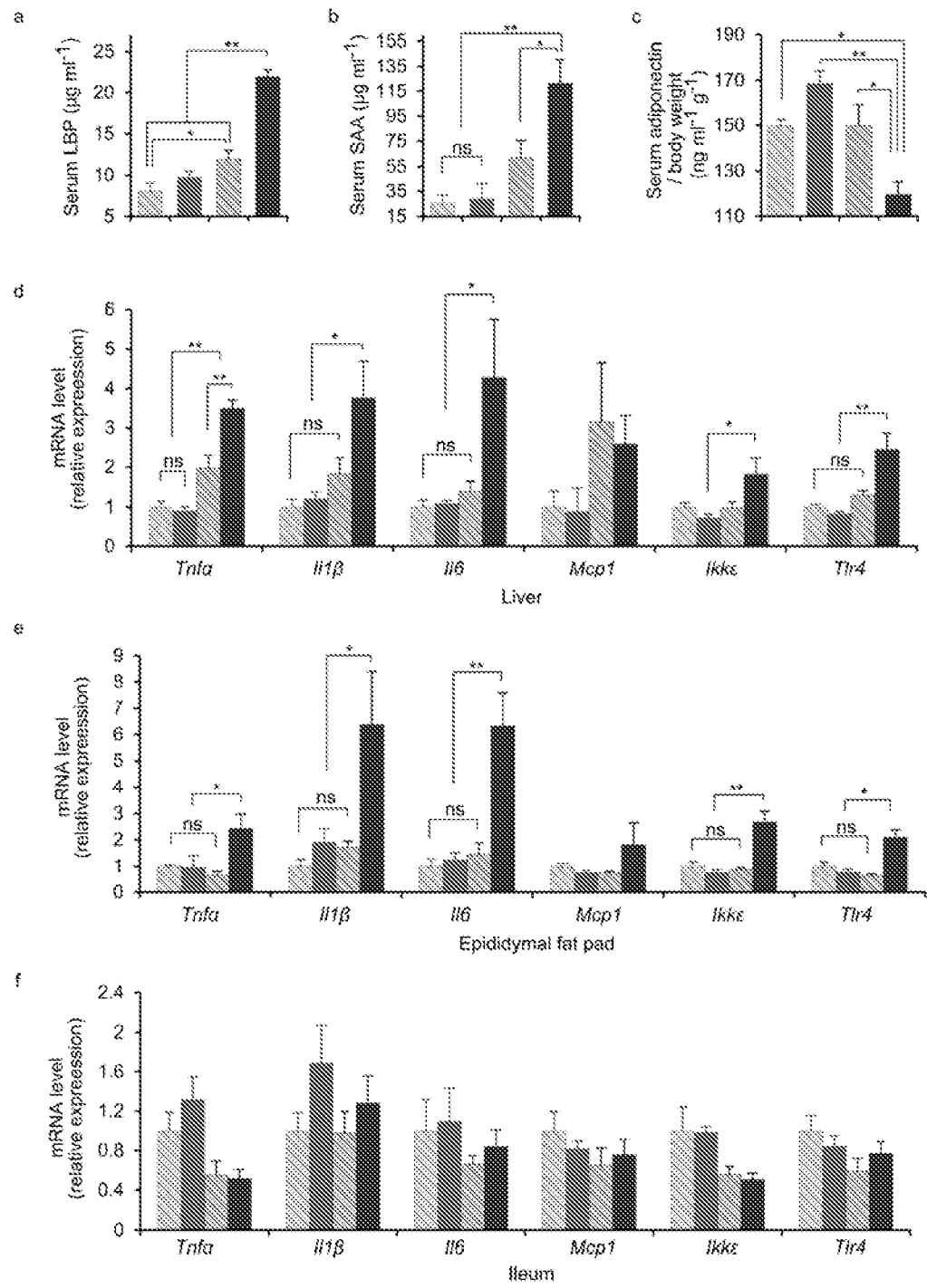


FIGURE 3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2013/080589

A. CLASSIFICATION OF SUBJECT MATTER

see the extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, CNPAT, CNKI, ISI Web of knowledge:

obesity, animal model, endotoxin, inoculating, colonization, high fat, HFD, metabolic disorder, enterobacter

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	CN 102792919 A (SHANGHAI JIAO TONG UNIVERSITY), 28 November 2012 (28.11.2012), see para [0004], [0011], [0012], [0014], [0019], example 1	8-44
PX	Na Fei and Liping Zhao, "An opportunistic pathogen isolated from the gut of an obese human causes obesity in germfree mice", The ISME Journal, 23 December 2012 (23.12.2012), vol. 7, no. 4, pages 880-884	8-32
A		33-44

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 25 October 2013 (25.10.2013)	Date of mailing of the international search report 21 Nov. 2013 (21.11.2013)
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Name and mailing address of the ISA/CN The State Intellectual Property Office, the P.R.China 6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088 Facsimile No. 86-10-62019451	Authorized officer LI, Yang Telephone No. (86-10)62411031
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2013/080589

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Patrice D Cani and Nathalie M Delzenne, "Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota", Current Opinion in Pharmacologh, 2009, vol. 9, no. 6, pages 737-743	8-44

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2013/080589

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 1-7
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-7 relate to a non-human obesity animal model. The subject matter of these claims is animal varieties, the case set forth in PCT Regulation 39.1 (ii)
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2013/080589

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
CN 102792919 A	28.11.2012	None	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2013/080589

CLASSIFICATION OF SUBJECT MATTER

A01K 67/02 (2006.01) i

A61K 49/00 (2006.01) i