Bifidobacterium longum ATCC BAA-999 (BL999) and weight control

The present invention generally relates to the field of weight management. The present invention relates for example to the use of *Bifidobacterium longum* ATCC BAA-999 to support weight management, promote weight loss and/or to treat obesity.

During the past decades, the prevalence of obesity has increased worldwide to epidemic proportion. Approximately 1 billion of people worldwide are overweight or obese, conditions that increase mortality, mobility and economical costs. Obesity develops when energy intake is greater than energy expenditure, the excess energy being stored mainly as fat in adipose tissue. Body weight loss and prevention of weight gain can be achieved by reducing energy intake or bioavailability, increasing energy expenditure and /or reducing storage as fat. Obesity represents a serious threat to health because it is associated with an array of chronic diseases, including diabetes, atherosclerosis, degenerative disorders, airway diseases and some cancers.

Modifications of the intestinal flora were recently associated with obesity. These changes were demonstrated in obese mice to affect the metabolic potential of gut microbiota resulting in an increased capacity to harvest energy from the diet (Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006; Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006). Such modifications of gut microbiota are proposed to contribute to the pathophysiology of obesity. Probiotics, the beneficial bacteria present in food or food supplements, are known to

modify the intestinal microbiota (Fuller R & Gibson GR. Modification of the intestinal microflora using probiotics and prebiotics. Scand J. Gastroenterol. 1997).

Some probiotics are known to have effects on obesity, however, these effects appear to be as rule strain specific.

For example, EP1974734 discloses the use of probiotic bacteria, for example Bifidobacterium longum ATCC BAA-999, capable of promoting the development of an early bifidogenic intestinal microbiota in the manufacture of a medicament or therapeutic nutritional composition for reducing the risk of development of overweight or obesity of an infant later in life. Infants are children under the age of 12 months, and effects are seen at the age of 4.

It would be desireable to have available a probiotic strain that has an immediate weight-loss effect, in particular in adults.

Consequently, it was the object of the present invention to improve the state of the art and in particular to provide the art with a composition comprising an bacterial strain that is effective, readily available, low priced and safe to administer without unwanted side effects which can be used to support weight management and to treat or prevent obesity and which has an immediate effect, in particular in adults.

This object is achieved by the subject matter of the independent claims.

The present inventors have found that - unexpectedly - the strain *Bifidobacterium longum* ATCC BAA-999 (BL999) achieves this object.

Hence, the inventors unexpectedly found that *Bifidobacterium longum* ATCC BAA-999 (BL999) cannot only be used in infants to prevent obesity later in life, but also to treat or prevent obesity and associated metabolic disorders and/or to support weight management in adults, while having an immediate effect.

An immediate effect means an effect on body weight in about 1-4 weeks, for example in an experimental setup as described in the examples.

Bifidobacterium longum ATCC BAA-999 (BL999) may be obtained commercially from specialist suppliers, for example from Morinaga Milk Industry Co. Ltd. of Japan under the trade mark BB536.

Bifidobacterium longum ATCC BAA-999 (BL999) may be cultured according to any suitable method. It may be added to products in a freeze-dried or spray-dried form, for example.

One embodiment of the present invention is a composition comprising *Bifidobacterium longum* ATCC BAA-999 for supporting weight loss and/or weight maintenance in mature humans and/or animals.

The present invention also relates to the use of *Bifidobacterium longum* ATCC BAA-999 for the preparation of a composition to support weight loss and/or weight maintenance in mature humans and or animals.

A further embodiment of the present invention is the use of *Bifidobacterium longum* ATCC BAA-999 for the preparation of a composition to promote weight loss.

Still, a further embodiment of the present invention is the use of *Bifidobacterium longum* ATCC BAA-999 for the preparation of a composition to support weight management.

The compositions described in the framework of the present invention are in particular beneficial for long term applications. The inventors have shown in an animal model that a mouse treated with the composition described in the present invention will put on significantly less weight than a control mouse if fed a high caloric diet. In fact, a mouse treated with the composition of the present invention will in fact loose weight. While the observed effect was immediate, that is to say was observed within a week, this effect was the more pronounced the longer the composition was administered. The experiment was continued for about two weeks and the observed effects increased with time.

Consequently, in a preferred embodiment of the present invention, the composition is to be administered for at least 1 weeks, at least 2 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, and/or at least 8 weeks.

In this specification, the following terms have the following meanings:

The term "*Bifidobacterium longum* ATCC BAA-999" is meant to include the bacterium, a cell growth medium with the bacterium or a cell growth medium in which *Bifidobacterium longum* ATCC BAA-999 was cultivated. *Bifidobacterium longum* ATCC BAA-999 may be present as viable bacteria, as non-replicating bacteria, or as a mixture thereof.

"Mature" is the age or stage when an organism can reproduce. In humans, the process of maturing is termed puberty.

"Body mass index " or "BMI" means the ratio of weight in Kg divided by the height in metres, squared.

"Overweight" is defined for an adult human as having a BMI between 25 and 30.

"Obesity" is a condition in which the natural energy reserve, stored in the fatty tissue of animals, in particular humans and other mammals, is increased to a point where it is associated with certain health conditions or increased mortality. "Obese" is defined for an adult human as having a BMI greater than 30.

"Probiotic" means microbial cell preparations or components of microbial cells with a beneficial effect on the health or well-being of the host. (Salminen S, Ouwehand A. Benno Y. et al "Probiotics: how should they be defined" Trends Food Sci. Technol. 1999:10 107-10).

"Prebiotic" means food substances intended to promote the growth of probiotic bacteria in the intestines.

"Food grade bacteria" means bacteria that are used and generally regarded as safe for use in food.

"Weight loss" in the context of the present invention is a reduction of the total body weight. Weight loss may for example refer to the loss of total body mass in an effort to improve fitness, health, and/or appearance.

"Weight management" or "weight maintenance" relates to maintaining a total body weight. For example, weight

management may relate to maintaining a BMI in the area of 18,5-25 which is considered to be normal.

The compositions of the present invention may for example be administered to overweight or obese humans or animals.

The composition of the present invention may contain protective hydrocolloids (such as gums, proteins, modified starches), binders, film forming agents, encapsulating agents/materials, wall/shell materials, matrix compounds, coatings, emulsifiers, surface active agents, solubilising agents (oils, fats, waxes, lecithins etc.), adsorbents, carriers, fillers, co-compounds, dispersing agents, wetting agents, processing aids (solvents), flowing agents, taste masking agents, weighting agents, jellifying agents, gel forming agents, antioxidants and antimicrobials. The composition may also contain conventional pharmaceutical additives and adjuvants, excipients and diluents, including, but not limited to, water, gelatine of any origin, vegetable gums, ligninsulfonate, talc, sugars, starch, gum arabic, vegetable oils, polyalkylene glycols, flavouring agents, preservatives, stabilizers, emulsifying agents, buffers, lubricants, colorants, wetting agents, fillers, and the like. In all cases, such further components will be selected having regard to their suitability for the intended recipient.

The composition may be a nutritionally complete formula.

The composition according to the invention may comprise a source of protein.

Any suitable dietary protein may be used, for example animal proteins (such as milk proteins, meat proteins and egg proteins); vegetable proteins (such as soy protein, wheat

protein, rice protein, and pea protein); mixtures of free amino acids; or combinations thereof. Milk proteins such as casein and whey, and soy proteins are particularly preferred.

The proteins may be intact or hydrolysed or a mixture of intact and hydrolysed proteins. It may be desirable to supply partially hydrolysed proteins (degree of hydrolysis between 2 and 20%), for example for animals believed to be at risk of developing cows' milk allergy. If hydrolysed proteins are required, the hydrolysis process may be carried out as desired and as is known in the art. For example, a whey protein hydrolysate may be prepared by enzymatically hydrolysing the whey fraction in one or more steps. If the whey fraction used as the starting material is substantially lactose free, it is found that the protein suffers much less lysine blockage during the hydrolysis process. This enables the extent of lysine blockage to be reduced from about 15% by weight of total lysine to less than about 10% by weight of lysine; for example about 7% by weight of lysine which greatly improves the nutritional quality of the protein source.

The composition may also contain a source of carbohydrates and a source of fat.

If the composition includes a fat source, the fat source preferably provides 5% to 40% of the energy of the composition; for example 20% to 30% of the energy. A suitable fat profile may be obtained using a blend of canola oil, corn oil and high-oleic acid sunflower oil.

A source of carbohydrates may be added to the composition.

The source of carbohydrates preferably provides about 40% to 80% of the energy of the composition. Any suitable

carbohydrate may be used, for example sucrose, lactose, glucose, fructose, corn syrup solids, maltodextrins, and mixtures thereof. Dietary fibre may also be added if desired. Dietary fibre passes through the small intestine undigested by enzymes and functions as a natural bulking agent and laxative. Dietary fibre may be soluble or insoluble and in general a blend of the two types is preferred. Suitable sources of dietary fibre include soy, pea, oat, pectin, guar gum, gum Arabic, fructooligosaccharides, galacto-oligosaccharides, sialyl-lactose and oligosaccharides derived from animal milks. A preferred fibre blend is a mixture of inulin with shorter chain fructo-oligosaccharides. Preferably, if fibre is present, the fibre content is between 2 and 40 g/l of the composition as consumed, more preferably between 4 and 10 g/l.

The composition may also contain minerals and micronutrients such as trace elements and vitamins in accordance with the recommendations of Government bodies such as the USRDA

One or more food grade emulsifiers may be incorporated into the composition if desired; for example diacetyl tartaric acid esters of mono- and di-glycerides, lecithin and mono- and di-glycerides. Similarly suitable salts and stabilisers may be included.

The composition is preferably orally or enterally administrable; for example in the form of a powder for re-constitution with milk or water.

Preferably, the composition is provided in the form of a powder, e.g., a shelf stable powder. Shelf stability can be obtained, for example by providing the composition with a water activity smaller than 0.2, for example in the range of 0.19-0.05, preferably smaller than 0.15.

Water activity or a_w is a measurement of the energy status of the water in a system. It is defined as the vapour pressure of water divided by that of pure water at the same temperature; therefore, pure distilled water has a water activity of exactly one.

The composition described above may be prepared in any suitable manner. For example, it may be prepared by blending together the protein, the carbohydrate source, and the fat source in appropriate proportions. If used, the emulsifiers may be included at this point. The vitamins and minerals may be added at this point but are usually added later to avoid thermal degradation. Any lipophilic vitamins, emulsifiers and the like may be dissolved into the fat source prior to blending. Water, preferably water which has been subjected to reverse osmosis, may then be mixed in to form a liquid mixture. The temperature of the water is conveniently about 50°C to about 80°C to aid dispersal of the ingredients. Commercially available liquefiers may be used to form the liquid mixture. The liquid mixture is then homogenised; for example in two stages.

The liquid mixture may then be thermally treated to reduce bacterial loads, by rapidly heating the liquid mixture to a temperature in the range of about 80°C to about 150°C for about 5 seconds to about 5 minutes, for example. This may be carried out by steam injection, autoclave or by heat exchanger; for example a plate heat exchanger.

Then, the liquid mixture may be cooled to about 60°C to about 85°C; for example by flash cooling. The liquid mixture may then be again homogenised; for example in two stages at about 10 MPa to about 30 MPa in the first stage and about 2 MPa to

about 10 MPa in the second stage. The homogenised mixture may then be further cooled to add any heat sensitive components; such as vitamins and minerals. The pH and solids content of the homogenised mixture are conveniently adjusted at this point.

The homogenised mixture is transferred to a suitable drying apparatus such as a spray drier or freeze drier and converted to powder. The powder should have a moisture content of less than about 5% by weight.

Bifidobacterium longum ATCC BAA-999 may be cultured according to any suitable method and prepared for addition to the composition by freeze-drying or spray-drying for example. Appropriate culturing methods for *Bifidobacterium longum* ATCC BAA-999 are known to those skilled in the art. Alternatively, bacterial preparations can be bought from specialist suppliers already prepared in a suitable form for addition to food products such as nutritional and infant formulas. The probiotic bacteria may be added to the formula in an appropriate amount, preferably between 10^2 and 10^{12} cfu/g powder, more preferably between 10^7 and 10^{12} cfu/g powder.

In one embodiment of the present invention the humans to be treated with the composition of the present invention are at least 16 years old.

If the animals to be treated with the composition of the present invention are dogs or cats, for example, the dog or cat may be at least 2 years old.

The composition may be a pharmaceutical composition. In a pharmaceutical composition the dosage of Bifidobacterium

longum ATCC BAA-999 can be carefully adjusted according to a doctor's recommendation.

The composition may also be a food product or a drink. As a food product the beneficial effects of Bifidobacterium longum ATCC BAA-999 would be available to everyone. Bifidobacterium longum ATCC BAA-999 could thus be easily used by everybody to support weight management, for example. Treatment or prevention of obesity could be initiated at a much earlier stage. Further, in a food product Bifidobacterium longum ATCC BAA-999 would be even more pleasant to consume. Examples of food products that are applicable to the present invention are yoghurts, milk, flavoured milk, ice cream, ready to east desserts, powders for re-constitution with, e.g., milk or water, chocolate milk drinks, malt drinks, ready-to-eat dishes, instant dishes or drinks for humans or food compositions representing a complete or a partial diet intended for pets or livestock.

Consequently, the composition according to the present invention may a food product intended for humans, pets or livestock.

In particular the composition may be intended for animals selected from the group consisting of dogs, cats, pigs, cattle, horses, goats, sheep, or poultry.

In a preferred embodiment the composition is a food product intended for adult species, in particular human adults.

The composition of the present invention may also comprise at least one other kind of other food grade bacteria or yeast. The food grade bacteria may be probiotic bacteria and are

preferably selected from the group consisting of lactic acid bacteria, bifidobacteria, propionibacteria or mixtures thereof.

Probiotic bacteria may be any lactic acid bacteria or Bifidobacteria with established probiotic characteristics. For example they may be also capable of promoting the development of a bifidogenic intestinal microbiota. Suitable probiotic Bifidobacteria strains include *Bifidobacterium lactis* CNCM I-3446 sold *inter alia* by the Christian Hansen company of Denmark under the trade mark Bb12, the strain of *Bifidobacterium breve* sold by Danisco under the trade mark Bb-03, the strain of *Bifidobacterium breve* sold by Morinaga under the trade mark M-16V and the strain of *Bifidobacterium breve* sold by Institut Rosell (Lallemand) under the trade mark R0070. A mixture of suitable probiotic lactic acid bacteria and Bifidobacteria may be used.

As food grade yeast the following can be used for example: Saccharomyces cerevisiae and/or Saccharomyces boulardii.

In a preferred embodiment of the present invention the composition further contains at least one prebiotic. Prebiotics can promote the growth of certain food grade bacteria, in particular of probiotic bacteria, in the intestines and can hence enhance the effect of Bifidobacterium longum ATCC BAA-999. Furthermore, several prebiotics have a positive influence on, e.g., digestion.

Preferably the prebiotic is selected from the group consisting of oligosaccharides and optionally contain fructose, galactose, mannose, soy and/or inulin; dietary fibers; or mixtures thereof.

The compositions may contain at least one prebiotic in an

amount of 0.3 to 10 % dry weight of the composition. Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health. Such ingredients are non-digestible in the sense that they are not broken down and absorbed in the stomach or small intestine and thus pass intact to the colon where they are selectively fermented by the beneficial bacteria.

Further examples of prebiotics include certain oligosaccharides, such as fructooligosaccharides (FOS) and galactooligosaccharides (GOS). A combination of prebiotics may be used, such as 90% GOS with 10% short chain fructo-oligosaccharides such as the product sold under the trade mark Raftilose® or 10% inulin such as the product sold under the trade mark Raftiline®.

A particularly preferred prebiotic is a mixture of galacto-oligosaccharide(s), N-acetylated oligosaccharide(s) and sialylated oligosaccharide(s) in which the N-acetylated oligosaccharide(s) comprise 0.5 to 4.0% of the oligosaccharide mixture, the galacto-oligosaccharide(s) comprise 92.0 to 98.5% of the oligosaccharide mixture and the sialylated oligosaccharide(s) comprise 1.0 to 4.0% of the oligosaccharide mixture. This mixture is hereinafter referred to as "CMOS-GOS". Preferably, a composition for use according to the invention contains from 2.5 to 15.0 wt% CMOS-GOS on a dry matter basis with the proviso that the composition comprises at least 0.02 wt% of an N-acetylated oligosaccharide, at least 2.0 wt% of a galacto-oligosaccharide and at least 0.04 wt% of a sialylated oligosaccharide.

Suitable N-acetylated oligosaccharides include

GalNAcα1,3Galβ1,4Glc and Galβ1,6GalNAcα1,3Galβ1,4Glc. The N-acetylated oligosaccharides may be prepared by the action of glucosaminidase and/or galactosaminidase on N-acetyl-glucose and/or N-acetyl galactose. Equally, N-acetyl-galactosyl transferases and/or N-acetyl-glycosyl transferases may be used for this purpose. The N-acetylated oligosaccharides may also be produced by fermentation technology using respective enzymes (recombinant or natural) and/or microbial fermentation. In the latter case the microbes may either express their natural enzymes and substrates or may be engineered to produce respective substrates and enzymes. Single microbial cultures or mixed cultures may be used. N-acetylated oligosaccharide formation can be initiated by acceptor substrates starting from any degree of polymerisation (DP) from DP=1 onwards. Another option is the chemical conversion of keto-hexoses (e.g. fructose) either free or bound to an oligosaccharide (e.g. lactulose) into N-acetylhexosamine or an N-acetylhexosamine containing oligosaccharide as described in Wrodnigg, T.M.; Stutz, A.E. (1999) Angew. Chem. Int. Ed. 38:827-828.

Suitable galacto-oligosaccharides include Galβ1,6Gal, Galβ1,6Galβ1,4Glc Galβ1,6Galβ1,6Glc, Galβ1,3Galβ1,3Glc, Galβ1,3Galβ1,4Glc, Galβ1,6Galβ1,6Galβ1,4Glc, Galβ1,6Galβ1,3Galβ1,4Glc Galβ1,3Galβ1,6Galβ1,4Glc, Galβ1,3Galβ1,3Galβ1,4Glc, Galβ1,4Galβ1,4Glc and Galβ1,4Galβ1,4Galβ1,4Glc. Synthesised galacto-oligosaccharides such as Galβ1,6Galβ1,4Glc Galβ1,6Galβ1,6Glc, Galβ1,3Galβ1,4Glc, Galβ1,6Galβ1,6Galβ1,4Glc, Galβ1,6Galβ1,3Galβ1,4Glc and Galβ1,3Galβ1,6Galβ1,4Glc, Galβ1,4Galβ1,4Glc and Galβ1,4Galβ1,4Galβ1,4Glc and mixtures thereof are commercially available under the trade marks Vivinal ® and Elix'or ®. Other suppliers of oligosaccharides are Dextra Laboratories, Sigma-Aldrich Chemie GmbH and Kyowa Hakko Kogyo Co., Ltd. Alternatively, specific

glycoslytransferases, such as galactosyltransferases may be used to produce neutral oligosaccharides.

Suitable sialylated oligosaccharides include NeuAcα2,3Galβ1,4Glc and NeuAcα2,6Galβ1,4Glc. These sialylated oligosaccharides may be isolated by chromatographic or filtration technology from a natural source such as animal milks. Alternatively, they may also be produced by biotechnology using specific sialyltransferases either by enzyme based fermentation technology (recombinant or natural enzymes) or by microbial fermentation technology. In the latter case microbes may either express their natural enzymes and substrates or may be engineered to produce respective substrates and enzymes. Single microbial cultures or mixed cultures may be used. Sialyl-oligosaccharide formation can be initiated by acceptor substrates starting from any degree of polymerisation (DP) from DP=1 onwards.

One advantage of the present invention is that Bifidobacterium longum ATCC BAA-999 are effective, both, as living bacterium as well as inactivated bacterial species. Consequently, even conditions that will not allow the presence of living bacteria will not abolish the effectiveness of Bifidobacterium longum ATCC BAA-999.

It is preferred, however that at least some of the Bifidobacterium longum ATCC BAA-999 are alive in the composition and preferably arrive alive in the intestine. This way they can colonize the intestine and increase their effectiveness by multiplication.

However, for special sterile food products or medicaments it might be preferable that Bifidobacterium longum ATCC BAA-999 are not alive in the composition. Hence, in one embodiment of

the present invention at least some of the Bifidobacterium longum ATCC BAA-999 are not alive in the composition.

Bifidobacterium longum ATCC BAA-999 will be effective in any concentration. If Bifidobacterium longum ATCC BAA-999 reaches the intestine alive, a single bacterium can theoretically be sufficient to achieve a powerful effect by colonization and multiplication.

However, for a pharmaceutical composition it is generally preferred that a daily dose of the medicament comprises between 10^2 and 10^{12} cfu of Bifidobacterium longum ATCC BAA-999. A particular suitable daily dose of Bifidobacterium longum ATCC BAA-999 is from 10^5 to 10^{11} colony forming units (cfu), more preferably from 10^7 to 10^{10} cfu.

In the case of inactivated Bifidobacterium longum ATCC BAA-999 it is generally preferred that a daily dose of the pharmaceutical composition comprises between 10^2 and 10^{12} cells of Bifidobacterium longum ATCC BAA-999. A particular suitable daily dose of Bifidobacterium longum ATCC BAA-999 is from 10^5 to 10^{11} cells, more preferably from 10^7 to 10^{10} cells.

For a food composition it is generally preferred that it comprises between 10^3 and 10^{12} cfu of Bifidobacterium longum ATCC BAA-999 per g of the dry weight of the food composition. A particular suitable amount of Bifidobacterium longum ATCC BAA-999 is from 10^5 to 10^{11} cfu per g of the dry weight of the food composition, more preferably from 10^7 to 10^{10} cfu per g of the dry weight of the food composition.

In the case of inactivated Bifidobacterium longum ATCC BAA-999 it is generally preferred that the food composition comprises between 10^3 and 10^{12} cells of Bifidobacterium longum ATCC BAA-

999per g of the dry weight of the food composition. A particular suitable amount of Bifidobacterium longum ATCC BAA-999 is from 10^5 to 10^{11} cells per g of the dry weight of the food composition, more preferably from 10^7 to 10^{10} cells per g of the dry weight of the food composition.

The daily dose of Bifidobacterium longum ATCC BAA-999 in a composition will depend on the particular person or animal to be treated. Important factors to be considered include age, body weight, sex and health condition.

For example a typical daily dose of Bifidobacterium longum ATCC BAA-999 in a composition will be in the range of 10^4-10^{12} cfu and/or cells per day, preferably 10^6-10^{10} cfu and/or cells per day, preferably 10^7-10^9 cfu and/or cells per day.

A further use of a composition comprising Bifidobacterium longum ATCC BAA-999 according to the present invention is to support weight loss and/or weight maintenance.

Since establishing and maintaining a proper body weight and - in particular - an acceptable weight percentage of fat in the body is a key step to treat or prevent metabolic disorders, a further use of a composition comprising Bifidobacterium longum ATCC BAA-999 according to the present invention is to treat or prevent metabolic disorders.

In particular, a composition comprising Bifidobacterium longum ATCC BAA-999 according to the present invention can be used to treat or prevent diabetes, hypertension and/or cardiovascular diseases and can hence make a significant contribution to the well-being of today's population in a number countries, in particular, in well developed countries.

Hence, the metabolic disorder may be selected from the group consisting of diabetes, hypertension, cardiovascular diseases, and combinations thereof.

The composition of the present invention may be for use in the treatment or prevention of metabolic disorders.

It is clear to those skilled in the art that any features described in this specification can be combined freely without departing from the scope of the present invention as disclosed.

Further features and advantages of the present invention result from the following Examples and Figures:

Figure 1 shows a reduction of body weight in mice treated with B. lungum ATCC BAA-999 (white square) as compared to control mice (black triangle).

Figure 2 shows a lower weight gain at week 7 (white panel) in mice receiving B. lungum ATCC BAA-999 as compared with control mice.

Figure 3 show an induction of the level of phosphorylated AMP kinase (pAMPk) skeletal muscle of mice treated with B. lungum ATCC BAA-999 as compared with control mice.

EXAMPLE

Weight change in AKR mice fed a high fat diet was followed for 7 weeks. During the last 2 weeks, mice (n=10/group) were randomly allocated to daily probiotic gavage with B. longum ATCC BAA-999 (10^9 cfu/day) or placebo (MRS). Mice receiving the probiotic experience a decrease in weight gain compared to

those gavaged with MRS (Figure 1). The weight gain measured at week 7 (Figure 2, white panel) was lower in treated mice as compared with control mice (Figure 2). Metabolic analysis in skeletal muscle revealed increased pAMPk activity in mice treated with B. *longum* ATCC BAA-999 (Figure 3). The data suggest that Bifidobacterium *longum* ATCC BAA-999 has significant effects on weight control through induction of metabolic effects on adipose and skeletal muscle tissues.

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

TO: **NESTEC S.A.** **AVENUE NESTLE 55** **CH-1800 VEVEY** NAME AND ADDRESS OF DEPOSITOR	RECEIPT IN THE CASE OF AN INITIAL DEPOSIT issued pursuant to Rule 7.1 by the INTERNATIONAL DEPOSITORY AUTHORITY identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: NCC 2818	Accession number issued by the INTERNATIONAL DEPOSITORY AUTHORITY: **CNCM I-3446**
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified in section I was accompanied by: ☒ a scientific description ☒ a proposed taxonomic designation (indicate as applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depository Authority accepts the microorganism identified in section I, which was received by it on **June 7, 2005** (date of the initial deposit)[1]	
IV. RECEIPT OF A REQUEST FOR CONVERSION	
This International Depository Authority received the microorganism identified in section I on (date of the initial deposit)[1] and received a request for conversion of the initial deposit into one conforming to the Budapest Treaty on (date of receipt of the request for conversion)	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: **COLLECTION NATIONALE DE CULTURES DE MICROORGANISMES [FRENCH NATIONAL COLLECTION OF MICROORGANISM CULTURES] (CNCM)** Address: **INSTITUT PASTEUR** **25 RUE DE DOCTEUR ROUX** **75724 PARIS CEDEX 15**	Signature(s) of the person(s) having the power to represent the International Depository Authority or of authorised official(s): Georges WAGENER [signature] [Stamp of the CNCM} Date: **PARIS, September 9, 2005**

[1] Where Rule 6.4.d) applies, this date shall be the date on which the status of the International Depository Authority was acquired.

Claims

1. Composition comprising *Bifidobacterium longum* ATCC BAA-999 for use in supporting weight loss and/or weight maintenance in mature humans and/or animals.

2. Composition in accordance with claim 1, wherein the humans or animals are overweight or obese.

3. Composition in accordance with one of the preceding claims, wherein the humans are at least 16 years old and are preferably adults.

4. Composition in accordance with one of the preceding claims, wherein the composition is a pharmaceutical composition, a drink or a food product.

5. Composition in accordance with one of the preceding claims, wherein the composition comprises at least one other kind of other food grade bacteria and/or yeast.

6. Composition in accordance with claim 5, characterized in that the food grade bacteria are selected from the group consisting of lactic acid bacteria, bifidobacteria, propionibacteria or mixtures thereof.

7. Composition in accordance with one of the preceding claims, wherein the composition further contains at least one prebiotic.

8. Composition in accordance with claim 6 characterized in that the prebiotic is selected from the group consisting of oligosaccharides and optionally contains fructose, galactose, mannose, soy and/or inulin; dietary fibers; or mixtures thereof.

9. Composition in accordance with one of the preceding claims, wherein at least some of the *Bifidobacterium longum* ATCC BAA-999 are alive in the composition.

10. Composition in accordance with one of the preceding claims, wherein at least some of the *Bifidobacterium longum* ATCC BAA-999 are not alive in the composition.

11. Composition in accordance with claim 4-10 characterized in that the pharmaceutical composition comprises between 10^2 and 10^{12} cfu of *Bifidobacterium longum* ATCC BAA-999 per daily dose, or in that the pharmaceutical composition comprises between 10^2 and 10^{12} cells of *Bifidobacterium longum* ATCC BAA-999 per daily dose.

12. Composition in accordance with claim 4-10 characterized in that the food product comprises between 10^3 and 10^{12} cfu of *Bifidobacterium longum* ATCC BAA-999 per g of the dry weight of the food composition, or in that the food product comprises between 10^3 and 10^{12} cells of *Bifidobacterium longum* ATCC BAA-999 per g of the dry weight of the food composition.

13. Composition in accordance with one of the preceding claims, wherein the composition is for use in the treatment or prevention of obesity.

14. Composition in accordance with one of the preceding claims, wherein the composition is reducing fat mass gain.

15. Composition in accordance with one of the preceding claims, wherein the composition is for use in the treatment or prevention of metabolic disorders.