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(54) Benævnelse: **SAMMENSÆTNING TIL BEHANDLING AF EN PATOGEN, METABOLISK TARMMIKROBIOTATILSTAND OG AFLEDTE SYGDOMME**

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DESCRIPTION

[0001] The present invention relates to the use of a composition as a drug or medical nutrition product for the treatment of a pathogenic metabolic state of the intestinal microbiota caused by pathological dysbiosis of metabolic origin and/or for the prevention of diseases derived from this pathogenic state.

[0002] The microbiota is a group of microorganisms (bacteria, archaea, viruses and eukaryotes) which are specific to each individual. These microorganisms are found on the skin and in the mouth, but the greatest number is found in the digestive system which has 10^{12} cells per gram and as there are 1.5 to 2 kg of microbiota, the presence of different species can be counted in the millions, i.e., from 3.3 to 8 million according to experts, and all of the microorganisms can be counted in the billions. This microbiota contains more than 150 times more genes than the host genome.

[0003] The microorganisms of the intestinal microbiota are classed by domain (for example bacteria), phylum (for example *Firmicutes*), class (for example *Clostridia*), order (for example *Clostridiales*), family (for example *Christensenellaceae*), genus (for example *Christensenella*) and species (for example *Christensenella minuta*).

[0004] Each individual has their own microbiota which comes from their history and their roots.

[0005] However, in a cohort, 75% of the species are found in 50% of the cohort and 57% are found in 90% of the cohort. In addition, 85% of the species are common between Europe, the USA and Japan.

[0006] The intestinal microbiota is not homogeneous. For the same individual it varies in quantity and quality from the stomach (10^1 with *lactobacillus, vellonella,* and *helicobacter*) to the duodenum, jejunum, ileum (10^3 to 10^7 with *bacilli, streptococcaceae, actinobacteria, actinomycinaeae,* and *corynebacteriaeae*) and finally the colon (10^{12} with *lachnospiraceae* and *bactériodetes*). Certain bacteria (*clostridium, lactobacillus,* and *enterococcus*) are also found in the mucus which lines the intestinal wall. The large phyla are relatively stable in an individual and the differences are at the species level, often a few percent of the total microbiota. Thus, pathogenic states are difficult to detect since they originate from these particular species.

[0007] Host anomalies (genetic and environmental) are found in an unbalanced pathogenic flora called pathogenic dysbiosis; the threshold of imbalance is difficult to fix as are the variations in genera and species unless there is a biomarker of the anomaly or the disease.

[0008] In recent years, it has become known that the intestinal microbiota is not only involved in digestion and the transformation of food into energy but that it plays a major role in maintaining good health and in the development of several diseases. Recent research suggests that the behavior of the brain, the entero-endocrine or neuro-vegetative system or even degenerative diseases are controlled by the microbiota.

[0009] It is also known that the intestinal microbiota is a major regulator of immunity and is intensely involved in regulating gene expression of the host.

[0010] Due to dysbiosis, i.e., a change in the composition of the microbiota, the microbiota can be associated with derived pathological metabolic illnesses such as type-2 diabetes or even cardiovascular diseases. Furthermore, some components of the microbiota have been associated with other diseases such as degenerative and intestinal diseases and some cancers.

[0011] The intestinal microbiota is also in direct correlation with the weight of the host. In fact, excess weight with comorbidities and obesity, defined by a body mass index (BMI) of 25 to 29.9 for excess weight and more than 30 for obesity, are associated with a pathological dysbiosis of metabolic origin of the intestinal microbiota.

[0012] Excess weight with comorbidities, obesity and metabolic diseases in the wider sense create problems in modern society whether in developed countries, emerging countries or in developing countries.

[0013] Researching the reasons for this epidemic has become of crucial importance so as not to experience a fall in life expectancy in the next 25 years and above all to avoid widening the span of bad health and dependency at the end of life.

[0014] The root causes of weight gain are an imbalance in food intake, in particular of saturated fat, fructose and carbohydrates associated or not with a significantly sedentary lifestyle in relation to food intake, i.e., in relation to the amount of energy. However, everyone is not equal when it comes to the same food intake. Indeed, there are differences in particular the level of absorbing and storing energy in the form of fat, and these differences are related to the pathogenic state of the intestinal microbiota.

[0015] In the case of excess weight and obesity, it has been noted that:

- the richness and the diversity of the microbiota is reduced
- some pathogenic families, genera and species take precedence over others
- the pathogenic microbiota has the capacity to extract more energy from food even indigestible fibers.

[0016] In addition, for those who are overweight or obese, calorie restriction together with dietary rebalancing increases the overall richness of the flora by the loss of fatty mass but there

is a difference in the results of the diet depending on the richness of the initial flora. For poor initial microbiota, the results are inferior, in particular in reducing inflammation and insulin resistance.

[0017] Furthermore, the analysis of thin or even anorexic people compared to obese people has established a correlation with certain microorganisms.

[0018] There has been research on a correlation between the phyla and excess weight/obesity and a relationship has been found between the two largest phyla: the relative ratio of *Firmicutes* to *Bacteriodetes* would increase with the BMI and the effect on weight loss would be reflected by the decrease in this ratio (Damms-machado a 2015, Pekkala s 2015, Remely m 2015, eslinger aj 2014, Bervoets I 2013, Fava f 2013), but this finding has also been contradicted by results demonstrating the opposite (Rahat-Rozenbloom, 2014 Xu P2012, Sefcíková Z 2010).

[0019] It is known that the composition of the microbiota of an individual is relatively homogenous over time at the level of the phyla (except temporary incidents such as antibiotic treatment) but that the genera can very, and it is also known that between several individuals, the phyla vary depending on age, geolocation, ethnicity, and lifestyle, etc. It is therefore impossible to establish a standard microbiota for good health, but it is possible research which genera and species indicate a pathological state or a non-pathological state.

[0020] Some enterotypes with a blend of positive or negative connections have been determined. In particular, three enterotypes are dominant in the world according to the preeminence of the most significant phylum: bacteriodetes, provotella and ruminococcus; however, it has not been possible to establish a direct correlation between these enterotypes and the metabolic diseases.

[0021] For the constitution of the microbiota up to 2012, it was accepted that the microbiota was built up by external influence and that it was fully acquired between the ages of 0 and 3 years from the environment and diet. In a study from November 2014, an international team led by Ruth Ley (Cornell University NY) published an article ("Human genetic shape the gut microbiome" Cell, 6 November, 159 789-799) under the direction of Julia Goodrich that contradicts the single acquisition concept by introducing the notion of inheritability for some families, genera and species; she made this observation by studying a cohort of identical and fraternal twins and control persons. Strains of the intestinal microbiota which have been demonstrated to be inheritable are:

- families of bacteria in the *Firmicutes: lachanospiraceae, ruminococcaceae* and *christensenellaceae,*
- archaea in the *Methanogens*: M. smithii.

[0022] The family *Christensenellaceae* is the most transmissible family. It comprises in particular the genus *Christensenella* and particularly the species *Christensenella minuta* which has been defined and cultivated recently by Masami Morotomi in Tokyo and described in the study "Description of christensenella minuta gen. nov.sp.nov. isolated from human faeces, which forms a distinct branch in the other clostridiales, and proposal of Christensenellaceae" (M Morotomi 2012 inter. Jour. Of systematic and evolution microbiology 62 144-149).

[0023] In children, at birth, the richness of *Christensenellaceae* is 20% in the meconium in comparison with 8.6% for the mother. This proportion then decreases relatively (to be approximately 5%) due to the rapid increase in other phyla, in particular those which will become predominant such as *Firmicutes, Bactériodetes, Proteobacteria* and *Actinobacteria* with the introduction of food (for example animal fat or proteins) and the environment.

[0024] The presence of *Christensenella* is associated with a low BMI as well as a state of "good health". The greater the relative amount of *Christensenella*, the more the host has a tendency to be lean and healthy. *Christensenella* would become, due to its low relative presence, the biomarker of pathogenic dysbiosis, a pathological metabolic state leading in particular to excess weight with comorbidities, obesity, and metabolic disease.

[0025] The family *christensenellaceae* is the leader in a "consortium" composed of the following families which are classed in order of their decreasing significance on metabolic effect: *dehalobacteriaceae* (firmicutes), unclassified *clostridiales*, unclassified genera *tenericutes* ML615J-28 and RF39, *methanobacteriaceae* (euryarchaeota), unclassified genus *firmicutes SHA-98*, *peptococcaceae*, and *verrucomicrobiaceae*.

[0026] The family *Christensenellaceae* is often positively linked to the abundance of *Methagenes* which is itself correlated with butyrate and propionate production, but *Christensenella* does not need this link to express itself in metabolism because it contributes directly to the phenotype of the host with whom it is associated.

[0027] Julia Goodrich ("Human genetic shape the gut microbiome" Cell, 6 November, 159 789-799) has continued her observation in germ-free mice seeded with obese microbiota in two groups, of which only one group received an implantation of *Christensenella*; its presence results in a weight gain of less than 30%.

[0028] The genus *Christensenella* is therefore recognized as a marker of leanness when a sufficient amount is present in the intestinal microbiota. On the other hand, it is a marker of excess weight and obesity when a low amount is present in the intestinal microbiota. The relative increase in *Christensenella* is the guarantor of weight loss, in particular in lean mass, and the engine of metabolic improvement.

[0029] In addition, these *Christensenella* bacteria are believed to be involved with other metabolic diseases such as diabetes, heart and vascular diseases, atherosclerosis, degenerative bone diseases, neurodegenerative diseases, cancers linked to metabolism, autoimmune diseases and metabolic steatosis and steatohepatitis.

[0030] Currently, there is a need for a product which is cable of acting on the intestinal microbiota in order to be able to be used for the treatment of the pathogenic microbiota and consequently for the prevention and treatment of derived metabolic diseases such as excess weight with comorbidities and obesity, etc.

[0031] To respond to this need, the present invention proposes using a composition which is capable of increasing the relative amount of *Christensenella* in the intestinal microbiota.

[0032] Such a composition is already known for different applications. Thus in patents FR 2981544 and FR 2981545, its use for reducing visceral fat and deep subcutaneous fat in humans and reducing visceral fat in patients suffering from obesity prior to bariatric surgery, respectively, is described.

[0033] Patent application FR3007985 also discloses a composition comprising a whey hydrolysate, alpha lactalbumin, a pharmaceutical active principle and a nutritional regulator. However, such a composition is not sufficient for the treatment of a pathological state of the microbiota.

[0034] To this end, the invention relates to the use of a composition comprising a mixture of active principles consisting of at least:

- a whey hydrolysate having a molecular weight of between 200 and 10,000 Daltons,
- a whey isolate and/or concentrate having a molecular weight between 15,000 and 20,000 Daltons, and
- calcium caseinate,

for use as a drug or medical nutrition product in humans for the treatment of a pathological state of the microbiota, namely a metabolic pathological dysbiosis, characterized by a drop in *Christensenella* in the intestinal microbiota.

[0035] The composition can also be used for the prevention or the treatment of at least one derived metabolic disease, in particular a metabolic disease which is signified by a deficiency of *Christensenella* in the intestinal microbiota and selected from excess weight with comorbidities, obesity, diabetes, heart and vascular diseases, atherosclerosis, neurodegenerative diseases, metabolism-related cancers, autoimmune diseases, steatosis, metabolic steatohepatitis and chronic inflammatory bowel diseases. The composition treats the pathological microbiota in particular by relatively increasing the bacterial genus

Christensenella (phylum *Firmicutes*, class *Clostridia,* order *Clostridiales,* family *Christensenellaceae,* genus *Christensenella*) in the intestine.

[0036] Advantageously, such a composition is capable of relatively increasing the amount of *Christensenella* in the intestinal microbiota in order to combat a metabolic pathogenesis. It can thus be used as a drug or medical nutrition product for rebalancing the intestinal microbiota by increasing the genus *Christensenella* and preventing and fighting diseases resulting from this pathological imbalance of the intestinal microbiota.

[0037] The invention is now described in detail, the description of the device being established with reference to the appended drawings, in which:

- Fig. 1 shows a diagram of the results of Table 1 relating to the relative abundance of *Christensenella*,
- Fig. 2 shows a barcode table, produced by E. Prifti, of results obtained from the study carried out on the intestinal microbiota of patients treated with a placebo and patients treated according to the invention.

[0038] The invention therefore has the object of providing a composition comprising a mixture of active principles consisting of at least:

- a whey hydrolysate having a molecular weight of between 200 and 10,000 Daltons,
- a whey isolate and/or concentrate having a molecular weight between 15,000 and 20,000 Daltons, and
- calcium caseinate,

for use as a drug or medical nutrition product in humans for the treatment of pathological dysbiosis, characterized by a deficiency of *Christensenella* in the intestinal microbiota and/or for the prevention or the treatment of at least one metabolic disease derived from this pathological state of the intestinal microbiota selected from excess weight with comorbidities, obesity, diabetes, heart and vascular diseases, atherosclerosis, neurodegenerative bone diseases, neurodegenerative diseases, metabolism-related cancers, autoimmune diseases, steatosis, metabolic steatohepatitis and chronic inflammatory bowel diseases.

[0039] The composition acts by relatively increasing the bacterial genus *Christensenella* in the intestine.

[0040] The composition is known but, surprisingly and unexpectedly, it acts on the microbiota and in particular is capable of increasing *Christensenella*.

[0041] Within the meaning of the invention, "deficiency" of *Christensenella* in the intestinal microbiota is understood to mean a level of *Christensenella* in the intestinal microbiota representing less than 0.01% of the total bacterial genera detected in the microbiota of a given

individual (collected in the stool, the abundance preferably being measured by a FISH sequencing or qPCR or meta-analysis test).

[0042] "*Christensenella*" is understood to mean the bacteria of the phylum *Firmicutes*, class *Clostridia,* order *Clostridiales,* family *Christensenellaceae,* and genus *Christensenella.*

[0043] Within the meaning of the invention, "medical nutrition composition" or "medical nutrition product" or "medical food" or foods for special medical purposes (FSMP) or dietary foods for special medical purposes (DFSMP) is understood to mean a food intended for therapeutic prevention or treatment, used alone or in combination with other therapies. It means a nutritional compound responding to a particular clinical situation and capable of constituting the exclusive or partial diet of the patient for whom it is intended.

[0044] Within the meaning of the invention, "whey concentrate" is understood to mean a whey extract obtained from a concentration of whey.

[0045] Within the meaning of the invention, "pathological dysbiosis" is understood to means a pathological state, characterized by an imbalance in the distribution of bacteria of the intestinal microbiota at the level of phyla, classes, orders, families, genera or species, leading to a risk of other diseases of which the origin is metabolic.

[0046] Within the meaning of the invention, "whey hydrolysate" is understood to mean any molecule or mixture of molecules obtained by a method comprising a step of the chemical hydrolysis or enzymatic hydrolysis of whey.

[0047] Within the meaning of the invention, "whey isolate" is understood to mean a whey extract which contains less than 1% of lactose and fatty substances.

[0048] Within the meaning of the invention, "drug" is understood to mean a product which has obtained marketing authorization as a product for the treatment of a disease.

[0049] Within the meaning of the invention, "microbiota" is understood to mean the intestinal microbiota.

[0050] The whey hydrolysate of the composition according to the invention has a molecular weight of between 200 and 10,000 Daltons, preferably between 200 and 3,500 Daltons. It consists substantially of dipeptides and tripeptides.

[0051] Preferably, it is a whey peptide hydrolysate comprising at least 90% peptides by weight of dry matter of the hydrolysate.

[0052] The whey isolate and/or concentrate preferably have a molecular weight between 15,000 and 20,000 Daltons.

[0053] The whey isolate is preferably produced from fresh milk and from dairies which do not pasteurize the milk in order to avoid the destruction of beta-lactoglobulin and alpha-

lactalbumin and extract the whey by ultrafiltration or microfiltration (filter size 0.1 mm). The isolate obtained by ion exchange is less suitable due to its low content of beta-lactoglobulin and alpha-lactalbumin. The isolate contains less than 1% of lactose and fatty substances and its peptide concentration is preferably at least 90% by weight of dry matter.

[0054] The whey concentrate is preferably obtained from a cheese whey which has not been pasteurized and contains beta-lactoglobulin, alpha-lactalbumin and glycomacropeptides. The peptide concentration in the concentrate is preferably at least 80% by weight of dry matter.

[0055] In addition, the calcium caseinate used in the composition according to the invention preferably has a molecular weight of between 20,000 and 35,000 Daltons.

[0056] Preferably, the ratio by weight between the calcium caseinate and the mixture consisting of the whey hydrolysate and isolate and/or concentrate is from 0.8 to 1.2 in the composition.

[0057] According to a particularly suitable embodiment, the mixture of active principles of the composition also comprises a mixture of amino acids.

[0058] The amino acids present in the composition are preferably at least tryptophan, glutamine, leucine, arginine and/or taurine but the composition may contain other amino acids such as isoleucine, valine, phenylalanine or threonine. The composition according to the invention very preferably comprises at least tryptophan, leucine, arginine and taurine.

[0059] When tryptophan is present, it should represent between 6 and 9%, preferably approximately 7%, by weight of the neutral amino acids present in the composition (leucine, isoleucine, valine, phenylalanine, tyrosine and tryptophan).

[0060] When arginine and taurine are present, the ratio of the weight of arginine to the weight of taurine is between 1.5 and 2.

[0061] In addition to the hydrolysate, whey isolate and/or concentrate and calcium caseinate and amino acids, the mixture of active principles of the composition according to the invention may comprise one or more elements selected from milk calcium, magnesium, vitamin B6, vitamin B9, vitamin E, vitamin D, zinc and chromium.

[0062] Furthermore, the composition may contain essential fatty acids, in particular omega 3. Preferably, the omega 3 is of vegetable origin with a high proportion of EPA.

[0063] According to a preferred embodiment, the mixture of active principles of the composition according to the invention comprises at least:

- 8 to 12% whey hydrolysate,
- 15 to 20% whey isolate/concentrate,
- 20 to 25% calcium caseinate,

the percentages being given by weight of dry matter of all the active principles present in the composition (apart from any excipients).

[0064] The composition may also contain freely added elements, such as amino acids, vitamins and minerals, which are added to the native constituents of whey hydrolysate, whey isolate, whey concentrate and calcium caseinate.

[0065] The composition according to the invention preferably consists of at least:

- 1.5 to 3% tryptophan,
- 12 to 20% branched amino acids,
- 6 to 10% aromatic amino acids,
- 0.8 to 1.5% taurine,
- 1.6 to 3% arginine,
- 1.2 to 3% milk calcium,
- 0.5 to 1% magnesium,
- 0.4 to 1% omega 3,
- 1 to 2 mg of vitamin B6 per 50 g of composition without excipients,
- 5 to 15 mg of zinc per 50 g of composition without excipients,
- 1 to 3 µg of vitamin D per 50 g of composition without excipients,
- 75 to 150 µg of chromium per 50 g of composition without excipients,
- 100 µg of vitamin B9 per 50 g of composition without excipients,
- 10 mg of vitamin E per 50 g of composition without excipients,

the percentages being given by weight of dry matter of all the active principles present in the composition (apart from any excipients), some of the constituents coming from the whey hydrolysate, the whey isolate, the whey concentrate and the calcium caseinate and the rest being freely added in the form of amino acids, vitamins and minerals.

[0066] The branched amino acids of the composition consist of leucine, isoleucine and valine, preferably:

- 50 to 60% leucine,
- from 18 to 25% isoleucine, and
- from 20 to 28% valine,

and the aromatic amino consist of tryptophan, phenylalanine and tyrosine, preferably:

- 15 to 24% tryptophan,
- from 38 to 46% phenylalanine, and
- from 35 to 43% tyrosine.

[0067] The composition according to the invention may be obtained by a method such the one described in the following:

- a first mixture is obtained by mixing the constituents in the following order: the calcium caseinate, whey isolate, whey concentrate, whey hydrolysate, free amino acids, magnesium, and milk calcium. The pH should be approximately 7 and stabilized at this level.
- adding a vitamins, minerals, and fatty acids to the first mixture.

[0068] In this way, a powder is obtained which can be transformed into tablets or liquid or even used in its powder form in sachets, sticks, tins or capsules, for example.

[0069] The composition according to the invention, when it is administered orally in a sufficient amount, makes it possible to modify the composition of the intestinal microbiota by relatively increasing the amount of *Christensenella* in the microbiota. The composition makes it possible to restore the metabolic functionality by relatively increasing *Christensenella* within the general population of the microbiota at least to a level representing 0.01% of the total of the bacterial genera detected in the microbiota of a given individual (collected in the stool, the abundance preferably being measured by a FISH sequencing or qPCR or meta-analysis test).

[0070] This increase makes it possible to fight pathological metabolic dysbiosis caused by a deficiency of *Christensenella* in the intestinal microbiota.

[0071] The increase in the relative amount of *Christensenella* also makes it possible to prevent or fight metabolic diseases of which the origin is a pathological metabolic dysbiosis, characterized by a relative deficiency of *Christensenella* in the intestinal microbiota, and in particular:

- excess weight with comorbidities
- obesity
- diabetes (and pre-diabetes)
- heart and vascular diseases
- atherosclerosis
- neurodegenerative bone diseases
- neurodegenerative diseases, in particular Parkinson's and Alzheimer's,
- metabolism-related cancers, in particular cancer of the esophagus, colon, endometrium, kidney and breast for women post menopause,
- autoimmune diseases
- steatosis, metabolic steatohepatitis and NASH (non-alcoholic steatohepatitis),
- chronic inflammatory bowel diseases.

[0072] The composition according to the invention is particularly useful for persons having a relative proportion of *Christensenella* of less than 0.01% of the total of the bacterial genera detected in the microbiota (collected in the stool, the abundance preferably being measured by a FISH sequencing or qPCR or meta-analysis test), and for persons having a relative proportion of *Christensenella minuta* in the microbiota of less than 0.005% of the total abundance of the intestinal flora, i.e., of the total of the bacterial species detected in the microbiota (collected in the stool, the abundance preferably being measured by a FISH sequencing or qPCR or meta-analysis test).

[0073] Different methods can be used to analyze the microbiota (collection generally being made in the stool):

- culture according to conventional methods of bacterial culture known to a person skilled in the art
- biological methods through the 16S rRNA marker
- sequencing either by the SANGER method with the Operational Taxonomic Units or by pyrosequencing at the 16S rRNA level (method used in the study on the composition)
- electrophoretic, polymorphic and ribosomal imprint
- DNA with microarray
- FISH (Fluorescence In Situ Hybridization) and qPCR (quantitative polymerase chain reaction) with amplification of a group
- meta-analysis with metagenomics (composition and function of all genes), metaproteomics (analysis of proteins), metabolomics (metabolic profile) and metatranscriptomics (through RNA).

[0074] According to an alternative embodiment, in extreme cases of medical emergency, it is possible for persons lacking in *Christensenella* to be implanted with bacteria of the genus *Christensenella* in the intestine prior to the administration of the composition according to the invention by the use of a probiotic which is specific to this bacterium in a capsule or by direct implantation in the jejunum by probe. The implantation should represent 75 to 100 mg of *Christensenella* bacteria. The implanted bacteria may be bacteria obtained by culturing bacteria of the genus *Christensenella* taken from the stool of said person treated if there are traces therein or bacteria obtained by culturing bacteria of the genus *Christensenella* taken from the stool of lean and healthy persons.

[0075] The use of a composition according to the invention in suitable amounts makes it possible, optionally with prior implantation, to bring the presence of *Christensenella* to a

minimum relative proportion of 0.01% compared to all the taxa of the microbiota collected in the stool. The composition could also make it possible to obtain a relative amount of *Christensenella minuta* (when the analytical method makes it possible to descend as far as the species) of at least 0.0075% (compared to the total of the taxa of the microbiota collected in the stool).

[0076] Surprisingly, the composition according to the invention is also capable of regulating, in the microbiota, bacteria families which are known for their significant relative presence in excess weight, obesity, and metabolic diseases and selected from the *clostridiales*, in particular *lachnospiraceae* and *clostridiaceae*.

[0077] Also astonishingly, it is also capable of regulating, in the microbiota, bacteria families which are known for their ability to increase the amount of energy withdrawn from food and selected from the bacteria which give off butyrate and propionate due to fermentation but also increase the energy by digesting fibers which are not normally digestible, specifically: *Roseburia*, *Faecalbacterium* (in particular the species *prausnitzii*), *Akkermansia* (in particular the species *municiphali*), *Eubacterium* (in particular the *rectal* species), *Methanobrevibacter* (in particular the species *smithii*). This additional transformation of energy is one of the most likely causes of the epidemic of excess weight and metabolic dysfunction. It is necessary to reduce these "energizers", but without reaching a deficiency, because the microbiota is a large consumer of butyrate, propionate and acetate (in order of importance); the genus *Akkermansia* and the species *Akkermansia municiphali* (which can also be inherited) are particularly important because they are the main producers of propionate which guarantees proper functioning of the intestinal barrier by limiting the passage of LPS; in fact, LPS which is induced by excessively fatty diets (saturated fats in particular) is the main thing responsible for metabolic dysfunction, hepatitis steatosis and the build-up of adipose tissue. The regulation by the invention of two genes which can be inherited and are difficult to evolve is essential for restoring the proper functioning of the metabolism and serves as more indication of the success of the intervention of the treatment by the invention.

[0078] The composition according to the invention can be provided in any form suitable for oral administration. It can in particular be provided in the form of a powder or granules, a ready-to-use beverage, food bars or extrudates, capsules, dispersible tablets or any suitable pharmaceutical form, the composition being added to with conventional excipients and fillers known to a person skilled in the art.

[0079] The daily dose of composition according to the invention (dose of mixture of active principles without the excipients) is preferably between 66 and 110 g, preferably in two doses

of 33 to 55 g distributed one in the morning at breakfast or 11 am as a snack, and one as an afternoon snack.

[0080] Advantageously, as part of a weight-loss objective, the bioavailability in the organism of the amino acids, peptides and proteins present in the composition in the organism is between 10 minutes and 5 hours, which makes an effect possible which is both fast and long-lasting so as to limit the amount of daily food intake.

[0081] In addition, the presence of milk calcium makes it possible to improve the palatability of the dietary product according to the invention by masking in particular the bitter taste of the whey hydrolysate such that it helps to eliminate the risk that people stop taking it for taste reasons and abandon their treatment before its term.

[0082] The composition according to the invention fits into personalized medicine and constitutes a great advancement in particular in pharmacometabolic medicine.

[0083] The composition according to the invention can in fact be used as a treatment which adapts to the metagenome of the person being treated.

[0084] Thus, before treatment by the composition according to the invention, a test may be carried out in order to detect the presence or not of *Christensenella* and its proportion in the microbiota. This test makes it possible to establish a probability of total or partial success or failure of the treatment. The test may be carried out again at the end of the treatment in order to analyze the level of increase in the bacteria, then again at one year in order to measure the sustainability of the treatment and the danger of relapse with the option of redoing the preventative treatment. The test may also involve other bacteria, in particular *Akkermansia*. The advantage is that the patient is no longer treated blindly and is followed for life in their chronic disease.

[0085] The invention also makes it possible to resolve the problems of therapeutic failures which are numerous whether through discontinuing the course of treatment most often at the beginning, or by significant weight gain after a few months or years. Failures are blamed on a lack of motivation or energy to make a permanent effort but the blockage may be due to the virtual absence or absence of *Christensenella* in the microbiota.

[0086] The invention is now illustrated by a non-limiting example of a dietetic composition, provided in the form of a powder of 55 g (active principles and excipients) packaged in a sachet.

[0087] This composition is obtained from the following active principles:

- 5g of whey hydrolysate having a molecular weight between 200 and 3,500 Daltons,
- 10 g of whey isolate/concentrate having a molecular weight between 15,000 and 20,000 Daltons,

- 13 g of calcium caseinate having a molecular weight between 20,000 and 35,000 Daltons,
- 1 mg of vitamin B6,
- 10 mg of zinc,
- 0.45 g of taurine,
- 40 µg of chromium,
- 10 mg of vitamin E,
- 100 µg of vitamin B9,
- 2.5 µg of vitamin D,
- 270 mg of omega 3 of vegetable origin,
- qsp 4,2 g of leucine,
- qsp 0.8 g pf tryptophan,
- qsp 0.9 g of arginine,
- qsp 0.75 g of milk calcium,
- qsp 0.36 g of magnesium.

[0088] "qsp X g" of an element of the composition is understood to mean the total amount of this element in the composition: amount provided by proteins (calcium caseinate, whey isolate, whey concentrate, whey hydrolysate) and supplemented by adding the element in free form to reach X g.

[0089] The invention is now illustrated by a study showing the effect of the composition on the intestinal microbiota, in particular on *Christensenella*.

[0090] The study involved 54 subjects. It is an ancillary study of a randomized blind international clinical study with another active product as a control. Two ancillary studies were carried out on 54 subjects, one on the microbiota and the other on adipose tissue.

[0091] The clinical study involved 108 patients whose characteristic was being overweight or obese with at least 3 factors of severe comorbidity; the subjects were included during a visit comprising the morphological criteria of a CT scan, a DEXA scan and a full biological analysis. For the ancillary study involving 54 subjects, a subcutaneous adipose tissue sample and a stool collection were also taken. The subjects received either the composition according to the invention or an isocaloric active control corresponding to the composition according to the invention but in which the whey was replaced by a pea protein known for its effect on the microbiota.

[0092] The study of the microbiota comprised taking one sample at the start and the other at the visit in week 12 at the end of the treatment of the product with moderate calorie restriction.

The stool was frozen and at the end of the study, it was analyzed by pyrosequencing. The analyzes were conducted by the main investigator, Institute of Cardiometabolism and Nutrition in Paris.

[0093] Of the 54 subjects, 27 were treated by the composition according to the invention (composition of the example) and 27 by the control product.

[0094] The stool was collected at the start and at the end of the treatment of 12 weeks with the two products and a calorie restriction of 600 kcal.

[0095] The results for the study of the microbiota are presented in Table 1 below and in Fig. 1 and 2.

[0096] For the results presented in Fig. 2, the following elements should be noted:

- the Wilcoxon test between genera was carried out on the basis of a normalized abundance,
- at the value $p < 0.05$, 16 genera are more abundant with the placebo treatment and 26 genera are more abundant with the treatment according to the invention,
- only one genus survives multiple tests: *Christensenella*.

Table 1:

	STANDARD *Artificial community of bacteria (1)*		INVENTION					CONTROL				
	Relative presence	READS	PR W0	PR W12	READS W0	READS W12	Δ%	PR W0	PR W12	READS W0	READS W12	Δ%
POPULATION					27							
TOTAL TAXA	100	82,394	100	100	75,875	74,220	-1.5	100	100	73,838	72,365	-1.2
FIRMICUTES	72.9	60,019	67.2	68.2	51,260	50,633	12.0	69.3	63.5	51,953	45,953	-7.4
BACTERIODETES	20.7	17,057	19.9	22.4	14,863	16,660	16	20.5	25.9	15,063	18,724	21.3
PROTEOBACTERIA	3.0	2,470	7	4.8	3,430	3,538	-20.6	5.8	6.8	4,281	4,962	16.5
ACTINOBACTERIA	5.0	4,148	2.3	1.7	1,721	1,264	-16.3	2.6	2.1	1,896	1,496	-12.3
FIRMICUTES/ BACTERIODETES		3.5	N		3.4	3.0				3.4	2.5	NS
CONSORTIUM OF EXCESS WEIGHT (2)	22	18,147	22.2	19.8	17,093	14,670	-9	25.3	25.0	18,730	18,110	0.5
ENERGIZERS CONSORTIUM (3)	5.2	4,290	8.7	8.2	6,592	6,073	-6	10.5	12.0	7,801	8,670	13
of which AKKERMANSIA	NC	NC	0.0161	0.0337	12	25	118	0.0097	0.0186	7	13.5	57%
CONSORTIUM OF CHRISTENSENELLACEAE (4)	11.5	9,494	11.4	11.4	8,389	8,486	1.6	0.07	11.0	8,982	8,630	-3.8
GENUS CHRISTENSENELLA	**0.0097**	**8**	**0.0028**	**0.013**	**2**	**10**	**380**	**0.002**	**0.0027**	**1.5**	**2**	**60%**

(continued)

	STANDARD *Artificial community of bacteria (1)*		INVENTION					CONTROL				
	Relative presence	READS	PR W0	PR W12	READS W0	READS W12	Δ%	PR W0	PR W12	READS W0	READS W12	Δ%
(1) there is no ideal microbiota model since it is individual and depends on genetics and those acquired by each person; for the study, a standard of cultivated families was developed; this standard was used to place the results in a context												
(2) the consortium of excess weight comprises many families in particular in the clostridiales order but two appear significant for their impact assessment number: lachnospiraceae and clostridiaceae												
(3) the consortium of "energizers" are the bacteria which give off butyrate and propionate due to fermentation but also increase energy by digesting fibers which are normally indigestible; it is composed of Roseburia, Faecalbacterium, Akkermansia, Eubacterium and Méthanobrevibacter. A balance in this group is particularly important.												
(4) the consortium of christensenellaceae comprises the families: Christensenelloceae, Méthanobacteriaceae, Dehalobacteriaceae, Peptococcaceae, Verrucomicrobiaceae, unclassified Clostridiales (the present study includes unclassified clostridiales and others which give weight to this family).												

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→ Regarding the total microbiota, it should be noted that there is a relative poverty of the two groups, Invention and Control, compared to the standard which is explained by the metabolic situation of these two groups; treatment does not improve this general situation, which is logical since 12 weeks is an insufficient amount of time for the large phyla.

→ Regarding the mass of the large traditional phyla firmicutes and bacteriodetes and their relationship, there is a relative poverty but especially for the firmicutes and this is explained by the situation of insulin resistance and very advanced inflammation; the situation improves slightly on average for the group treated by the composition according to the invention. Regarding the relationship (currently controversial), the situation improves slightly on average but more for the control group also through a deterioration of the firmicutes, which demonstrates that this relationship is of little interest.

→ In the other two large phyla, the *Proteobacteria* are high and the *Actinobacteria* are low; these results perfectly reflect the metabolic situation of these subjects. There is a favorable relative change for the group of the invention in the two phyla.

→ The consortium of the excess weight changes favorably for the invention but goes in the opposite direction for the control.

→ The consortium of the energizers which is high in the two groups is resized for the group of the invention unlike the control group; the *Akkermansia* which was particularly low is resized in the two groups but with a more substantial increase for the group of the invention.

→ The consortium of the *Christensenellaceae* (Goodrich 2013) increases in the groups treated according to the invention, reflecting the improvement at the metabolic level and on the weight loss.

→ In the study on the family *Christensenellaceae*, the unclassified genera and those not belonging to *Christensenella* are crucial (> 99%). According to a 2013 publication by Rajilic-Strojanovic, these unclassified genera would be involved in the inflammation of inflammatory bowel disease. Here, a relative decrease of 1.26 to 1.12 can be seen for a treatment according to the invention and an increase of 0.83 to 0.89 can be seen for the control. However, as there could be a number of genera in this unclassified part which could evolve in a divergent manner, it is impossible to draw any conclusions; it is wise to wait for new discoveries in these unclassified genera.

→ The analysis of the genus *Christensenella* is the central focus of this study on the microbiota.

[0097] *Christensenella* is a bacterium which can be inherited rather than acquired and is not sensitive to the diet, in this category it seems to be the most significant of the group. This implies that for people who have low levels of *Christensenella*, it is "vital" to restore a healthy

metabolism to increase genus rather than the entire microbiota with prebiotics or probiotics which can cause increases in undesirable classes such as methanogens or helicobacters. The invention has a targeted effect on bacteria which are useful for metabolism. The regulation of *Akkermansia* is second priority and then the consortium of weight and energizers.

[0098] In addition, *Christensenella* is a marker bacterium of "normal" weight from a metabolic point of view and a marker of good health therefore of a microbiota which functions regularly without pathogenicity both in terms of genetics and lifestyle. Furthermore, it has been shown by Goodrich that the implantation of *Christensenella* reverses the weight curve by reducing weight gain in mice, which in humans results in weight loss.

[0099] The effect of *Christensenella* is particularly essential for metabolic regulation in order to erase pathogenic factors which are reflected in metabolic diseases.

[0100] The genus *Christensenella* is relatively under-represented in the two groups (0.0028 and 0.002 vs. 0.0097) compared to the standard, which confirms the decrease in this genus in a population suffering from metabolic problems. The effect of the two products increases *Christensenella* from 0.0028% to 0.014% for the group of the invention (380%) with a significance compared to the start ($p = 0.002$), while the control goes from 0.002% to 0.0028% (+60% and not significant). In 12 weeks, the group treated according to the invention very clearly passes above the standard (0.014% vs. 0.0097%) and it is significant compared to the control (0.004).

[0101] In addition, it should be noted that of all the genera which comprise the human microbiota (1,500 known to this day), the only significant genus in the study of the invention that stands out from all the statistical tests is the *Christensenella* which appears to be one of the most significant genera of pathological metabolic states of the microbiota and derived metabolic diseases.

[0102] In addition, the clinical results and the ancillary study on the adipose tissue are present in Table 2 below:

Table 2:

	Composition according to the invention	
	FAS TOTAL Base Line	FAS TOTAL Δ%
CLINICAL STUDY		
POPULATION	108	

VISCERAL FATTY MASS CT Scan cm^2	192.3	-10.4%
TOTAL FATTY MASS CT Scan cm^2	489.7	-8.9%
FATTY MASS DXA kg	37.0	-8.9%
LEAN MASS DXA kg	51.2	-0.1%
WAIST MEASUREMENT cm	104.5	-4.2%
WEIGHT kg	92	-3.9%
TAS mm	131.7	-2.7%
TAD mm	81.6	-4.0%
GLYCEMIA mmol	5.5	-2.8%
INSULINEMIA mUI/l	12.6	-19.0%
HOMA IR	3.1	-20.5%
HOMA B	124	-9.3%
HOMA S	59.9	+40.6%
TOTAL CHOLESTEROL mmol	5.2	-7.2%
HDL mmol	1.2	+0.2%
LDL mmol	3.2	-7.6%
TRIGLYCERIDES mmol	1.6	-9.6%
TNF pg/ml	1.9	-10.2%
ADIPOSE TISSUE ANCILLARY STUDY		
GLYCEROL pg/ml	16	-20.1%
IL6	3,707	-13.3%
0^2 ENERGY CONSUMPTION µM	0.002	+63%
ADIPONECTIN pg/ml	11,426	+13.3%

[0103] It should be noted that the composition used according to the invention has a certain effect on the metabolic function of the adipocyte which results in the increase of adiponectin in the adipose tissue which reflects the two basic dysfunctions at the origin of many metabolic and degenerative diseases:

- inflammation with a drop in IL6 in the adipose tissue and TNFα; this is explained by the activation of p105 on adipocytes which regulates NPκB, the modulator of inflammatory cytokines
- insulin resistance which is improved by the decrease in insulinemia, the decrease in HOMA IR, but above all by a very noticeable improvement in the sensitivity of insulin by HOMA S
- two other functions on adipose tissue should be noted: the regulation of lipolysis of adipocytes in people who are overweight (glycerol) and the increase in energy production (O^2 consumption)
- improvement of comorbidity factors such as blood pressure, glycemia, insulinemia, cholesterol, LDL and triglycerides is both a consequence of metabolic improvement but also the direct effect of active compounds of the invention, in particular amino acids
- finally, no serious side effects were noted during the treatment apart from a few temporary digestive problems.

[0104] According to the invention, it is therefore possible to act on a pathological dysbiosis of the intestinal flora by means of the relative increase in the genus *Christensenella*, and consequently to act on the metabolic diseases marked by this pathology. The effects on other families, genera or species reinforce the effect of *Christensenella*.

Patentkrav

1. Sammensætning, der omfatter en blanding af aktivstoffer, der består af mindst:

- et vallehydrolysat med en molekylvægt på mellem 200 og 10.000 Dalton,
- et valleisolat og/eller -koncentrat med en molekylvægt på mellem 15.000 og 20.000 Dalton og
- calciumcaseinat,

til anvendelse som lægemiddel eller medicinsk ernæringsprodukt til personer til behandling af en patologisk dysbiose i tarmmikrobiotaen, **kendetegnet ved** en deficiens af *Christensenella* i tarmmikrobiotaen.

2. Sammensætning til anvendelse ifølge et af de foregående krav hos personer, hos hvem slægten *Christensenella* tegner sig for mindre end 0,01 % af samtlige bakterieslægter, der er påvist i tarmmikrobiotaen.

3. Sammensætning til anvendelse ifølge krav 2, **kendetegnet ved, at** bakteriearten *Christensenella* er af bakterieslægten *Christensenella minuta.*

4. Sammensætning til anvendelse ifølge krav 3 hos personer, der har en relativ andel af *Christensenella minuta* i floraen på under 0,005 % af tarmfloraens totalforekomst.

5. Sammensætning til anvendelse ifølge et af kravene 3 eller 4 til regulering af bakterier i mikrobiotaen, der er kendt for deres evne til at øge mængden af energi udledt fra fødevarerne, og som er valgt blandt slægterne *Roseburia, Faecalbacterium, Akkermansia, Eubacterium, Methanobrevibacter.*

6. Sammensætning til anvendelse ifølge et af de foregående krav til personer med underskud af *Christensenella,* og hos hvem disse bakterier af slægten *Christensenella* forinden er implanteret i tarmen ved anvendelse af et specifikt probiotikum af denne bakterie i kapsel eller ved direkte implantation via sonde.

7. Sammensætning til anvendelse ifølge krav 6, **kendetegnet ved, at** de bakterier, der er implanteret i den behandlede person, er bakterier, der er opnået ved dyrkning af bakterier af

slægten *Christensenella,* der er udtaget fra den behandlede persons tarm.

8. Sammensætning til anvendelse ifølge krav 6, **kendetegnet ved, at** de bakterier, der er implanteret i den behandlede person, er bakterier, der er opnået ved dyrkning af bakterier af slægten *Christensenella,* hvis stamme er udtaget fra tarmen hos slanke og raske personer.

9. Sammensætning til anvendelse ifølge et af de foregående krav, **kendetegnet ved, at** calciumcaseinatet har en molekylvægt på mellem 20.000 og 35.000 Dalton.

10. Sammensætning til anvendelse ifølge et af de foregående krav, **kendetegnet ved, at** vallehydrolysatet har en molekylvægt på mellem 200 og 3500 Dalton.

11. Sammensætning til anvendelse ifølge et af de foregående krav, **kendetegnet ved, at** vægtforholdet mellem calciumcaseinat og blandingen bestående af vallehydrolysatet og isolatet og/eller -koncentratet ligger mellem 0,8 og 1,2.

12. Sammensætning til anvendelse ifølge et af de foregående krav, **kendetegnet ved, at** blandingen af aktivstoffer ligeledes omfatter en blanding af aminosyrer.

13. Sammensætning til anvendelse ifølge krav 12, **kendetegnet ved, at** blandingen af aminosyrer består af mindst én af aminosyrerne valgt blandt tryptophan, glutamin, leucin, arginin og taurin.

14. Sammensætning til anvendelse ifølge krav 13, **kendetegnet ved, at** tryptophan tegner sig for mellem 6 og 9 vægt-% af de neutrale aminosyrer, der er til stede i sammensætningen.

15. Sammensætning til anvendelse ifølge et af kravene 13 eller 14, **kendetegnet ved, at** vægtforholdet mellem arginin og vægten af taurin ligger mellem 1,5 og 2.

16. Sammensætning til anvendelse ifølge et af de foregående krav, **kendetegnet ved, at** blandingen af aktivstoffer ligeledes omfatter mindst ét af elementerne valgt blandt mælkecalcium, magnesium, vitamin B6, vitamin B9, vitamin E, vitamin D, zink og chrom.

17. Sammensætning til anvendelse ifølge et af de foregående krav, **kendetegnet ved, at** blandingen af aktivstoffer ligeledes omfatter essentielle fedtsyrer.

18. Sammensætning til anvendelse ifølge krav 17, **kendetegnet ved, at** de essentielle fedtsyrer er omega 3.

19. Sammensætning til anvendelse ifølge et af de foregående krav, **kendetegnet ved, at** vallehydrolysat tegner sig for mellem 8 og 12 %, valleisolatet og/eller –koncentratet for mellem 15 og 20 % og calciumcaseinatet for mellem 20 og 25 %, hvilke procentsatser er angivet i tørstofvægt af samtlige aktivstoffer, der er til stede i sammensætningen.

20. Sammensætning til anvendelse ifølge et af de foregående krav, **kendetegnet ved, at** den består af mindst:

- 1,5 til 3 % tryptophan,
- 12 til 20 % forgrenede aminosyrer,
- 6 til 10 % aromatiske aminosyrer,
- 0,8 til 1,5 % taurin,
- 1,6 til 3 % arginin,
- 1,2 til 3 % mælkecalcium,
- 0,5 til 1 % magnesium,
- 0,4 til 1 % omega 3,
- 1 til 2 mg vitamin B6 pr. 50 g sammensætning uden excipienser,
- 5 til 15 mg zink pr. 50 g sammensætning uden excipienser,
- 1 til 3 µg vitamin D pr. 50 g sammensætning uden excipienser,
- 75 til 150 µg chrom pr. 50 g sammensætning uden excipienser,
- 100 µg vitamin B9 pr. 50 g sammensætning uden excipienser,
- 10 mg vitamin E pr. 50 g sammensætning uden excipienser,

hvor procentsatserne er angivet efter tørstofvægt af samtlige aktivstoffer, der er til stede i sammensætningen.

21. Sammensætning til anvendelse ifølge et af de foregående krav, **kendetegnet ved, at** den fremstår i form af pulver eller granulater, drikkeklar drikkevare, fødevarebarrer eller -ekstrudater, kapsler eller gelatinekapsler eller dispergerbare tabletter.

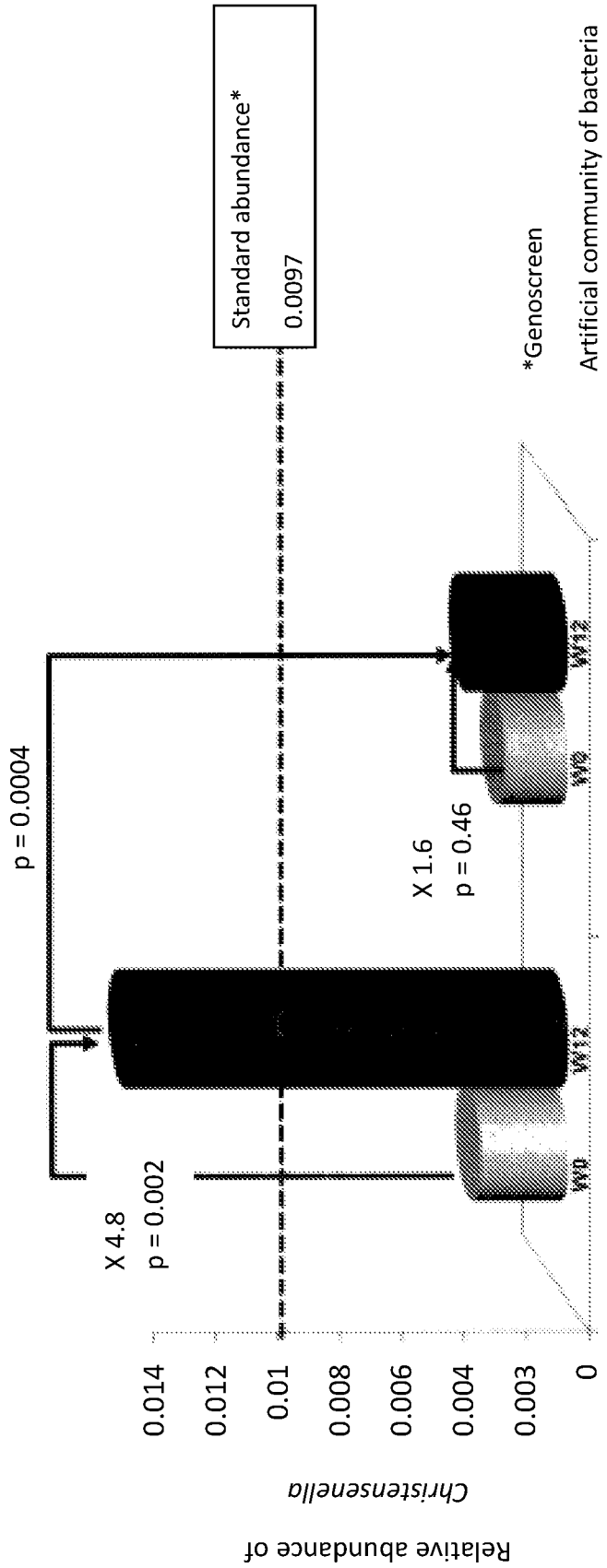
Relative abundance of
Christensenella
0.014
0.012
0.01
0.008
0.006
0.004
0.003
0
Standard abundance*
0.0097
*Genoscreen
Artificial community of bacteria
Invention
Control
W0
W12
W0
W12
X 4.8
p = 0.002
p = 0.0004
X 1.6
p = 0.46

Fig. 1

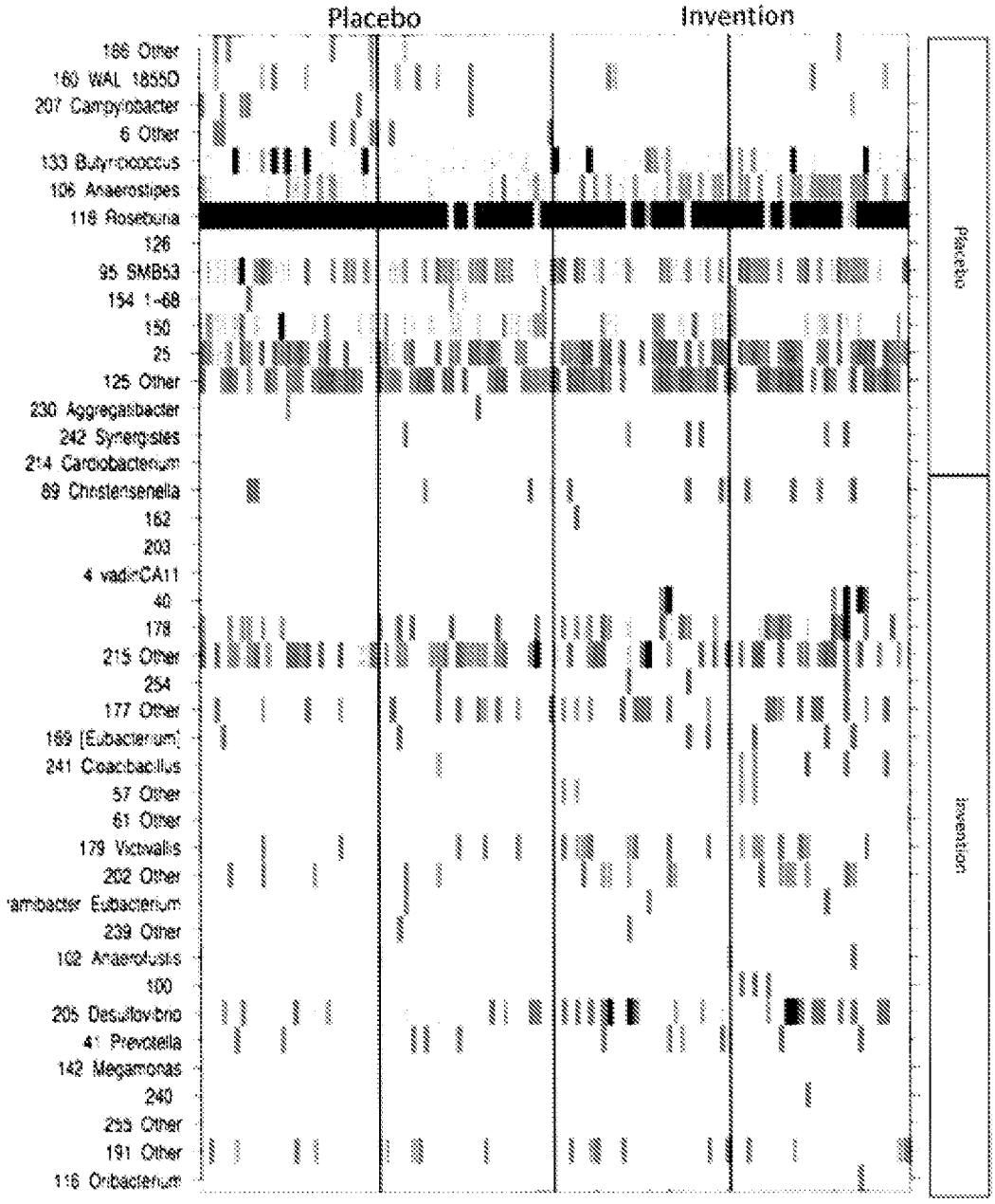
Placebo
Invention
186 Other
160 WAL 1855D
207 Campylobacter
6 Other
133 Butyricicoccus
106 Anaerostipes
118 Roseburia
126
95 SMB53
154 1-68
150
25
125 Other
230 Aggregatibacter
242 Synergistes
214 Cardiobacterium
89 Christensenella
162
203
4 vadinCA11
40
178
215 Other
254
177 Other
169 [Eubacterium]
241 Coxiella
57 Other
61 Other
179 Victivallis
202 Other
ambacter Eubacterium
239 Other
102 Anaerofustis
100
205 Desulfovibrio
41 Prevotella
142 Megamonas
240
253 Other
191 Other
116 Oribacterium
placebo
invention

Fig. 2