

(19) **DANMARK**

(10) **DK/EP 3164144 T3**



Patent- og
Varemærkestyrelsen

(12) Oversættelse af
europæisk patentskrift

- (51) Int.Cl.: **A 61 K 35/744 (2015.01)** **A 23 L 33/135 (2016.01)** **A 61 K 35/747 (2015.01)**
A 61 P 1/14 (2006.01) **A 61 P 3/00 (2006.01)** **C 12 R 1/25 (2006.01)**
- (45) Oversættelsen bekendtgjort den: **2021-09-27**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2021-07-28**
- (86) Europæisk ansøgning nr.: **15722225.8**
- (86) Europæisk indleveringsdag: **2015-05-15**
- (87) Den europæiske ansøgnings publiceringsdag: **2017-05-10**
- (86) International ansøgning nr.: **EP2015060753**
- (87) Internationalt publikationsnr.: **WO2015173386**
- (30) Prioritet: **2014-05-16 FR 1454422**
- (84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**
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- (54) Benævnelse: **Lactobacillus-sammensætning til stimulering af juvenil vækst hos mennesker og dyr ved mangelfuld ernæring**
- (56) Fremdragne publikationer:
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Composition allowing promoting the human and animal juvenile growth in cases of malnutrition

The present invention relates to a pharmaceutical or probiotic composition allowing promoting the human and animal juvenile growth in cases of malnutrition in mammals. This composition comprises as active principle or ingredient at least one bacterium with intestinal tropism, preferably a lactic acid bacterium.

Described as « an additional member », the intestinal microbial community (or intestinal microbiota) plays a key role beneficial for the host by exerting many biological functions, such as contributing to the efficiency of the digestion, the metabolism of the substrates, the pathogen control, or further the establishment and homeostasis of immune responses. The imbalances between different intestinal bacterial populations have sometimes deleterious repercussions leading to the development of diverse pathologies such as chronic inflammatory diseases or metabolic disorders including obesity or further type 2 diabetes. These imbalances are also potentially involved in the development of cancers and the establishment of behavioural syndromes. Maintaining the balance of the intestinal microbiota is therefore essential: influencing its composition and/or its activity would be a major asset for treating the aforementioned syndromes. In this spirit, the idea of using bacterial strains called “probiotic” bacterial strains to intervene in pathologies influenced by the microbiota makes perfect sense.

Defined in 2001 by the World Health Organisation (WHO) and the Food and Agriculture Organisation of the United Nations (FAO), probiotics are “living microorganisms, which, when they are ingested in a sufficient amount, exert positive effects on health, beyond traditional nutritional effects”.

The present invention, for its part, relates to the re-establishment of conditions allowing optimising the juvenile growth and restoring a normal juvenile growth in a context of malnutrition in mammals.

The present invention thus has the goal of proposing new compositions allowing promoting the juvenile growth or re-establishing a normal juvenile growth in mammals, humans or animals, having been or being subjected to a malnutrition, in particular an under-nourishment and/or poor assimilation.

Another object of the invention is to provide such compositions, based on the use of bacterial strains having an intestinal tropism, in particular commensal strains, or acceptable as a probiotic.

Another object of the invention is to provide such compositions which may also

be included in a therapeutic context.

Still another object of the invention is to offer a probiotic or therapeutic treatment method.

5 Still another object of the invention is to provide such compositions and methods allowing a health and/or nutritional claim in accordance with the legislation in force, particular European legislation.

10 The invention is based on the fact that some bacterial strains having an intestinal tropism in an animal species have an effect promoting or re-establishing the juvenile growth in a subject of this same species or of another species which is subject to a malnutrition, in particular to under-nourishment or poor assimilation. It was thus able to be demonstrated that bacterial strains of the *Lactobacillus* genus were capable of promoting the juvenile growth both in a *Drosophila* model and in a mouse model coupled with poor nutritional diets and furthermore a connection was able to be established between these results in mice and an increase in the serum IGF-1 level in mice treated under these conditions with bacteria.

15 The object of the present invention is therefore a composition comprising at least one *Lactobacillus* strain having an intestinal tropism selected from *L. plantarum* and *L. fermentum*, for use in promoting the juvenile growth in the treatment of malnutrition in mammals. The notion of malnutrition groups together under-nourishment, over-nourishment and poor assimilation. The invention concerns in particular under-nourishment and/or poor assimilation. It preferably concerns under-nourishment. The indication of the composition might be a therapeutic or a health indication, as a drug, or nutritional indication, as a probiotic.

20 The term bacterium having "intestinal tropism", means a bacterium with the capability of passing through the gastric barrier and which is capable of persisting in the intestine.

In accordance with an advantageous feature of the invention, the bacteria may promote the production of IGF-1 in humans or animals which are treated with the composition according to the invention.

30 Bacterial strains belonging to the following families: Lactobacillaceae, Streptococcaceae, Enterococcaceae, Leuconostocaceae, Bifidobacteriaceae are in particular described, from which one or more strains of the *Lactobacillus* genus, in particular one of the following species, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus casei*, *Lactobacillus paracasei*,
35 *Lactobacillus rhamnosus*.

More particularly, bacteria belonging to the species *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus* and in particular *L. casei* ATCC 393, *L. casei* L919, *L. paracasei* ATCC25302, *L. paracasei* Shirota, *L. rhamnosus* L900, *L. rhamnosus* L908, *L. rhamnosus* GG are described. According to the invention, the strain is selected from the species *Lactobacillus plantarum* and *Lactobacillus fermentum*. According to one embodiment of the invention, the bacterial strain is selected from *L. plantarum* WJL, *L. plantarum* G821, and *L. fermentum* ATCC9338.

In a specific embodiment, these are bacteria of the species *Lactobacillus plantarum*, for example the WJL strain or the G821 strain, deposited at the « National Collection of Cultures of Microorganisms (CNCM) » (Pasteur Institute) under the registration number CNCM I-4979 on May 11, 2015. The G821 strain was obtained by experimental evolution (i.e. by accumulation and selection of natural variants) of the strain *L. plantarum* NIZO2877.

The compositions according to the invention comprise at least one bacterial strain selected from the species *Lactobacillus plantarum* and *Lactobacillus fermentum*, which has the required properties and allows promoting or re-establishing a juvenile growth in spite of the malnutrition period. Of course, the composition according to the invention may comprise more than one bacterial strain meeting the needs of the invention. In particular, the composition comprises two or more of these bacterial strains, selected from a same species or from different species.

According to an interesting embodiment, the bacterium is an *L. plantarum*. Suitable strains are *L. plantarum* G821 and *L. plantarum* WJL (Eun-Kyoung Kim et al., Genome Announcements, November/December 2013, Vol. 1, no. 6 e00937-13, GenBank AUTE000000000, *Lactobacillus plantarum* WJL, whole genome shotgun sequencing project). This WJL strain was initially isolated and may be isolated from the *Drosophila* (JH Ryu et al., Science 2008, 319: 777-782).

Other examples of strains which are also described are the following: *L. casei* ATCC 393, *L. casei* L919 (Koryszewska-Baginska A. et al., September 26th, 2013, Genome Announc), *L. paracasei* ATCC25302, *L. paracasei* Shirota (Yuki N et al., Int J Food Microbiol. April 1st, 1999; 48(1): 51-7), *L. fermentum* ATCC9338, *L. rhamnosus* L900 (Aleksandrak-Piekarczyk T. et al., Genome Announc, August 15th, 2013), *L. rhamnosus* L908 (Koryszewska-Baginska A. et al., February 20th, 2014, Genome Announc.), *L. rhamnosus*GG (Kankainen M. et al., Proc. Natl. Acad. Sci. USA, October 6th, 2009).

The present invention therefore provides the technique with the teaching that bacterial strains with intestinal tropism promote the juvenile growth in particular in malnourished (human or animal) mammals. But the invention is not limited to this teaching, it further enables the person skilled in the art to safely determine the strains of bacteria useful for the invention. Different criteria may be based on tests, by being used alone or in association. Among these criteria, it will be noted that the serum IGF-1 level in the model animal (e.g. mouse), the larval growth in *Drosophila melanogaster*, the growth of model mice illustrated, for example, by the size of the femurs or the increase in size of the animals. Based on these criteria or similar criteria, it is possible for the person skilled in the art to develop tests comparing individuals reared in the presence or in the absence of the bacterium to be tested, under malnutrition conditions. The invention provides a significant addition by proposing axenic animal models and the rearing of the animals in the presence or in the absence of the bacterium or bacteria to be tested allowing concentrating the test on the analysis of the intrinsic properties of the tested strain(s) and thus overcoming the effect that the residing intestinal microbiota of conventional animals could have within the context of the functional test. This results in an increased predictivity of the test as to the functional potential of the tested strain.

The term "axenic" organism means an organism (e.g. a *Drosophila*, a mouse) reared in an environment devoid of microorganisms and therefore devoid of intestinal flora.

The term "monoxenic" organism means an associated axenic organism (e.g. *Drosophila*, mouse) reared in the presence of a single microorganism and therefore bearing this single microorganism as intestinal flora.

Within the context of the invention, the bacterial strains present in the compositions according to the invention are characterised by the fact they positively respond to, or were selected by using, a test in which the growth of larvae from axenic *Drosophila* on a yeast-deficient (and therefore protein-deficient) nutritive medium relative to a laboratory conventional nutritive medium. The laboratory conventional nutritional media generally comprise a *minima* a vegetable flour, typically corn flour, inactivated yeast, typically beer yeast or bread yeast (*Saccharomyces cerevisiae*), agar and water. An inactivated yeast-deficient medium does not allow an optimum post-embryonic (i.e. juvenile) development of *Drosophila* larvae, as this was reported in G. Storelli et al., Cell Metabolism 14, 403-414, 2011. A group of embryos is additionally seeded with the bacterium to be tested, which allows the larvae emerging from the embryos, of being associated with the bacterium to be tested. It is therefore possible

to detect whether this bacterium is likely to promote or allow the juvenile growth in spite of a yeast-deficient nutritive medium.

The *Drosophila* test is typically conducted in the following manner (Test A):

- 5 - a batch of embryos from axenic *Drosophila melanogaster* parents (e.g. the *Drosophila yw* strain, or any other strain called "wild" strain);
- on D1: these embryos are distributed into at least 2 groups (40 embryos, made in triplicate for a total of 120 embryos per group) on a nutritional medium comprising corn flour, agar and water, deficient in yeast, one of the groups of embryos is further inoculated with a suspension of about 10^8 CFU of the bacterium to be tested in a
10 saline buffer (e.g. PBS), which forms the monoxenic group, the other group forming the axenic group, the rearing is conducted at about 25°C until D7; typically the culture medium is constituted, for 1 litre of medium, of 7.14 g of agar, 80 g of corn flour and 6 g of inactivated yeast, the medium is cooked in boiling water for 10 min, then cooled; typically the same medium, non-deficient in inactivated yeast, contains 50 g
15 of inactivated yeast;
- on D7: at least 60 of *Drosophila* larvae of each group from the inoculated or noninoculated embryos (respectively monoxenic larvae and axenic larvae) are recovered, a fast thermal shock is applied thereto (e.g. 5 seconds; typically the larvae are placed for 5 seconds on a plate heated to 100°C; this means killing the larvae
20 without deforming them in order to allow measuring their size);
- the mean length of the larvae of each group is determined, and the obtained means are compared;
- the tested bacterial strain is considered as positively responding to the test if the mean of the length of the larvae of the monoxenic group is greater than the mean of
25 the length of the larvae of the axenic group with a p-value of less than or equal to 0.001, preferably less than or equal to 0.0001 in the Mann-Whitney statistical test, carried out on the entire data set of the sizes of the larvae of both groups.

The object of the present invention is therefore a composition comprising at least one *Lactobacillus* strain having an intestinal tropism, selected from the species
30 *Lactobacillus plantarum* and *Lactobacillus fermentum*, for use to promote the juvenile growth in the treatment of malnutrition in mammals, wherein the bacterial strain positively responds to the test on *Drosophila* as described above (Test A).

By way of examples, mention may be made of the following strains: *L. plantarum* WJL, *L. plantarum* G821, *L. fermentum* ATCC9338. Other strains may be
35 identified from bacteria with intestinal tropism and in particular from the species

mentioned above, and in particular *L. casei* ATCC 393, *L. casei* L919, *L. paracasei* ATCC25302, *L. paracasei* Shirota, *L. rhamnosus* L900, *L. rhamnosus* L908, *L. rhamnosus* GG.

In one embodiment of the invention, the WJL strain, or any other strain having a “marked” effect (for example *L. casei* ATCC 393, *L. fermentum* ATCC 9338, *L. paracasei* ATCC 25302) is used as a reference strain in order to identify and select the bacterial strains having a “marked” effect on the juvenile growth, namely an effect close to that of this reference strain, e.g. WJL (an effect not significantly different from the reference strain, e.g. WJL), or a “strong” effect on the juvenile growth (an effect significantly greater than the reference strain, e.g. WJL).

To this end, the *Drosophila* test (including 60 larvae per condition) is applied to the reference strain, e.g. WJL and to the strain to be tested (in parallel, preferably, or else previously generated reference data may be available for the reference strain, e.g. WJL, for example the data shown in the examples). The means obtained for both strains are then compared. The tested bacterial strain is considered as being a strain with a marked effect if the mean of the length of the larvae of the monoxenic group is not significantly different from the mean of the length of the larvae of the reference group, e.g. WJL with a p-value greater than 0.001 in the Mann-Whitney statistical test, carried out on the entire data set. The effect is strong if said mean for the strain to be tested is significantly greater than the mean for the reference strain, e.g. WJL, which is the case when the p-value of the statistical test is less than or equal to 0.001, preferably less than 0.0001. The effect is described as intermediate if said mean for the strain to be tested is significantly less than the mean for the reference strain, e.g. WJL, which is the case when the p-value of the statistical test is less than or equal to 0.001, preferably 0.0001.

The object of the present invention is therefore a composition comprising at least one *Lactobacillus* strain having intestinal tropism, selected from the species *Lactobacillus plantarum* et *Lactobacillus fermentum*, for use to promote juvenile growth in the treatment of malnutrition in mammals, in which the bacterial strain has a marked or strong effect on the growth in the *Drosophila* model as described above.

By way of examples, mention may be made of the following strains: *L. plantarum* WJL, *L. plantarum* G821, *L. fermentum* ATCC9338. Other strains may be identified from the bacteria with intestinal tropism and in particular from the species mentioned above, and in particular, *L. casei* ATCC 393, *L. casei* L919, *L. paracasei* ATCC25302, *L. paracasei* Shirota, *L. rhamnosus* L900, *L. rhamnosus* L908, *L.*

rhamnosus GG.

The object of the invention is also a composition comprising a bacterial strain with intestinal tropism of the *Lactobacillus* genus, selected from the species *L. plantarum* et *L. fermentum*, for a use aiming at promoting juvenile growth in the treatment of malnutrition in mammals, in which the *Lactobacillus* strain responds positively to the following test (Test D):

- a batch of embryos from axenic *Drosophila melanogaster* parents is available;
- on D1: these embryos are distributed into at least 2 groups (40 embryos, made in triplicate, i.e., 120 embryos per group) on a nutritional medium comprising corn flour, agar and water, deficient in yeast, one of the embryo groups is inoculated with a suspension of about 10^8 CFU of the bacterium in a saline buffer, which forms the monoxenic group test, the other group being inoculated with a suspension of about 10^8 CFUs of the reference bacteria *L. plantarum* WJL, *L. fermentum* ATCC 9338, in a saline buffer, forming the monoxenic reference group, the rearing of both groups is conducted at about 25°C up to D7;
- on D7: at least 60 *Drosophila* larvae of each group are recovered, a thermal shock is applied thereto;
- the mean of the length of the larvae of each of the groups is determined and the obtained means are compared;
- said bacterial strain having, relative to the reference bacterium, a strong effect with a mean of the length of the larvae of the monoxenic group test with this bacterial strain significantly higher than the mean for the monoxenic group with the reference strain, with a p-value less than or equal to 0.001, preferably 0.0001 in the Mann-Whitney statistical test, carried out on the entire data set of the sizes of the larvae of both groups.

According to the invention, it is also possible to determine whether a bacterial strain with intestinal tropism allows promoting growth in the case of malnutrition, by using an axenic mouse model which allows performing a follow-up of skeletal growth of mice in the presence of the bacterium to be tested in comparison with the absence of a microbiota and/or with the presence of a reference bacterial strain. This model may be used in first intention or on strains having positively responded to the *Drosophila* test.

According to a feature of the invention, the bacterial strains present in the composition according to the invention are characterised by the fact that they positively respond to the following skeletal growth test (Test B):

- from the same mouse line (typically Balb/c mice), a line of axenic parent mice and a line of monoxenic parent mice (associated with the bacterium to be tested) are established, and juveniles are produced which are reared with the parents in a conventional nutritional medium until they are weaned (on D21); in order to form the group of monoxenic juveniles, parents mono-associated with the bacterial strain to be tested are used,
- on D21: 8 weaned juveniles from each of these two lines, forming the monoxenic group and the axenic group are available, and are reared on a nutritional diet deficient in proteins (8%) and lipids (2.5%); typically the conventional nutritional diet comprises 23% of proteins and 5% of lipids,
- on D56: the mean of the sizes of the mice is determined for a relevant group by measuring from the tip of the snout to the base of the tail of each individual; another possible measurement consists of sacrificing the individuals, and of taking the femurs therefrom and measuring the latter.
- the strain of lactic acid bacteria being considered as positively responding to the test if the mean size of the individuals and/or femurs, of the monoxenic group, is greater than the mean size, respectively, of the individuals and/or femurs of the axenic group, with a p-value less than or equal to 0.05 in Tukey's statistical test

The object of the present invention is therefore a composition comprising at least one *Lactobacillus* strain having intestinal tropism, selected from the species *L. plantarum* and *L. fermentum*, for use to promote juvenile growth in the treatment of malnutrition in mammals, in which the bacterial strain positively responds to the skeletal growth test on mice (Test B). The *Drosophila* test allows a faster screening, such that generally, the test on *Drosophilas* is also available, and the bacterial strain positively responds to *Drosophila* and skeletal growth tests.

By way of example, mention may be made of the following strain: *L. plantarum* WJL. The strains *L. plantarum* G821 and *L. fermentum* ATCC9338 can also be mentioned. Other strains may be identified from bacteria with intestinal tropism and in particular from the species and strains mentioned above, and in particular from the strains *L. casei* ATCC 393, *L. casei* L919, *L. paracasei* ATCC25302, *L. paracasei* Shirota, *L. rhamnosus* L900, *L. rhamnosus* L908, *L. rhamnosus* GG.

In one embodiment of the invention, the strain WJL or another strain with a "marked" effect is used as a reference strain in order to identify and select bacterial strains having a "marked" effect on juvenile growth, namely an effect close to that of this reference strain, e.g. WJL (an effect not significantly different from the reference

strain, e.g. WJL), or a “strong” effect on juvenile growth (an effect significantly greater than that of the reference strain, e.g. WJL).

To this end, the mouse test (including 8 mice per condition) is applied to the reference strain, e.g. WJL and to the strain to be tested (in parallel, preferably or else reference data which are previously generated for the strain WJL may be available, for example data shown in the examples). The means obtained for both strains are then compared. The tested bacterial strain is considered as being a strain with a marked effect if the mean of the mean size of the individuals and/or of the femurs of the monoxenic group is not significantly different from the corresponding mean for the reference group, e.g. WJL with a p-value greater than 0.05 in Tukey’s statistical test. The effect is strong if said mean for the strain to be tested is significantly greater than the mean for the reference strain, e.g. WJL, which is the case when the p-value of the statistical test is less than or equal to 0.05. The effect is qualified as intermediate if said mean for the strain to be tested (which was qualified relative to the axenic mice in the preceding test) is significantly less than the mean for the reference strain, e.g. WJL, which is the case when the p-value of the statistical test is less than or equal to 0.05.

The composition according to the invention will preferably comprise at least one bacterial strain selected from the species *L. plantarum* and *L. fermentum* having such a marked or strong effect.

The bacterial strains according to the invention may be characterised by the fact that they have a positive impact on the serum IGF-1 level. This is how it was possible, based on a model of axenic mice, to show that mice reared in a medium with a low protein content but in the presence of the bacterium (monoxenic mice) had greater growth and at the same time a greater serum IGF-1 level, relative to these same mice reared with this protein-deficient medium and in the absence of the bacterium (axenic mice). This allows proposing a test allowing determining whether a bacterial strain has the potential of increasing the serum IGF-1 level, this test may be applied in association with a skeletal growth test in order to specify or refine the effect of the strain in this context of juvenile growth, or in a first intention.

In this case, the bacterial strains present in the composition according to the invention are characterised by the fact that they positively respond to the following serum IGF-1 level test (Test C):

- from the same mouse line (typically Balb/c mice), a line of axenic parent mice and a line of monoxenic parent mice (associated with the bacterium to be tested) are established, and juveniles are produced which are reared with the parents in a

conventional nutritional medium until they are weaned (on D21); in order to form the group of monoxenic juveniles, mono-associated parents with the bacterial strain to be tested are used,

- on D21: 8 weaned juveniles from each of these two lines are available, forming the monoxenic group and the axenic group, and are reared on a nutritional diet deficient in protein (8%) and lipids (2.5%); typically, the conventional nutritional diet comprises 23% of proteins and 5% of lipids,

- on D56: blood is taken from the juveniles of each group and the mean serum IGF-1 level is determined for each group; this measurement of the serum IGF-1 level is preferably made on diluted serum (1:25); commercial ELISA kits for detecting IGF-1 are used by following the instructions of the manufacturer,

- the strain of lactic acid bacteria is considered as positively responding to the test if the mean serum IGF-1 level of the monoxenic group is greater than the mean serum level of the axenic group with a p-value less than or equal to 0.05 in the Tukey statistical test.

The object of the present invention is therefore a composition comprising at least one *Lactobacillus* strain having intestinal tropism selected from the species *L. plantarum* and *L. fermentum*, for use to promote juvenile growth in the treatment of malnutrition in mammals, in which the bacterial strain increases the serum IGF-1 level (Test C). This is in particular a bacterial strain which positively responds to the test on mice as described above.

The object of the present invention is also a composition comprising at least one *Lactobacillus* strain having intestinal tropism selected from the species *L. plantarum* and *L. fermentum*, for use to promote juvenile growth in the treatment of malnutrition in mammals, in which the bacterial strain positively responds to the skeletal growth test on mice and increases the serum IGF-1 level in these same mice, in particular said strain positively responds to the test on *Drosophila*.

By way of example, mention may be made of the following strain: *L. plantarum* WJL. The strains *L. plantarum* G821 or *L. fermentum* ATCC9338 can also be mentioned. Other strains may be identified from bacteria with intestinal tropism and in particular from the species and strains mentioned above, in particular from the strains *L. casei* ATCC 393, *L. casei* L919, *L. paracasei* ATCC25302, *L. paracasei* Shirota, *L. rhamnosus* L900, *L. rhamnosus* L908, *L. rhamnosus* GG.

In one embodiment of the invention, the strain WJL or another strain with a

“marked” effect is used as a reference strain in order to identify and select bacterial strains having a “marked” effect on the serum IGF-1 level, namely an effect close to that of this reference strain, e.g. WJL (an effect not significantly different from the reference strain, e.g. WJL), or a “strong” effect on the serum IGF-1 level (an effect significantly greater than the reference strain, e.g. WJL).

To this end, the mice test (including 8 mice per condition) for measuring the serum IGF-1 level is applied to the reference strain, e.g. WJL and to the strain to be tested (in parallel, preferably, or else reference data which are previously generated for the reference strain may be available, e.g. WJL, for example the data shown in the examples). The means of serum IGF-1 levels obtained for both strains are then compared. The tested bacterial strain is considered as being a strain with a marked effect if the mean of the serum IGF-1 levels of the monoxenic group is not significantly different from the mean for the reference group, e.g. WJL with a p-value greater than 0.05, in Tukey’s statistical test. The effect is strong if said mean for the strain to be tested is significantly greater than the mean for the reference strain, e.g. WJL, when the p-value is less than or equal to 0.05. The effect is qualified as intermediate if said mean for the strain to be tested (previously qualified on the preceding axenic test) is significantly less than the mean for the reference strain, e.g. WJL, when the p-value is less than or equal to 0.05. The composition according to the invention will therefore preferably comprise a bacterial strain having such a marked or strong effect, and in particular this bacterial strain has a marked or strong effect both on skeletal growth and on the serum IGF-1 level.

The composition according to the invention, comprising at least one bacterial strain in accordance with the invention, is suitable for use as a drug intended to promote juvenile growth in the treatment of malnutrition in mammals characterised by protein deficiency. According to one feature of the invention, the subject to be treated, malnourished or having been malnourished, may have a serum IGF-1 level less than that encountered in individuals of the same age and gender and which are not malnourished.

The composition can be used in humans from birth to puberty.

The composition can be used in mammals, in particular productive livestock (cattle, sheep, goats, pigs, poultry), pets (dog, cat) and sport animals (horse, dromedary, camel), between weaning and sexual maturity.

The composition contains an amount from 10^5 to 10^{12} , in particular from 10^6 to 10^{12} , preferably from 10^8 to 10^{12} bacterial cells forming colonies (CFU) according to the

invention, per gram of composition. The term CFU means "colony forming units". The term "gram of composition" preferably means the pharmaceutical composition or the probiotic composition formed with bacteria, co-ingredients, and excipients or carriers. The term "bacterial cells" means a unique bacterial strain in accordance with the invention or a mixture of at least two bacteria, according to the invention.

The composition according to the invention comprises the lactic acid bacteria(um) in a living form. This may be a bacterial suspension, which may be frozen and thawed after use, or else a freeze-dried power, which may be used as such or after resumption in a suitable carrier. It may then comprise a conventional freeze-drying excipient.

The composition may be an oral administration form (for example a powder, a capsule, a tablet) optionally in a gastroprotected form allowing crossing the stomach and of releasing the bacteria in the intestine.

The compositions according to the invention are therefore useful for the therapeutic treatment to promote juvenile growth in the case of malnutrition of mammals, comprising the administration to a human (from birth to puberty) or animal (from weaning to sexual maturity) patient, in need thereof, that is to say malnourished or having undergone a malnutrition period, in particular with protein-deficiency. The patient in need thereof may have a serum IGF-1 level less than that encountered in individuals of the same age and gender and which are not malnourished.

As indicated, the method may be a therapeutic treatment comprises administration of a sufficient amount of a composition according to the invention as described above. The amount and the frequency of administration will in particular depend on the severity of the malnutrition, on the age and on the condition of the patient. The method will comprise the administration in one or several times, which may be staged over the growth period of the subject (up to puberty or sexual maturity), of doses of the composition according to the invention. The doses may be fractionated to facilitate the administration, in particular depending on the age of the patient. The administration frequency is in particular comprised between one dose (single or fractionated) daily and one dose monthly. Typically, the administration frequency will be comprised between one dose (single or fractionated) daily and one dose weekly, or even every 2, 3, 4, 5 or 6 days. Each dose (single or fractionated) represents in particular several grams to several tens of grams of composition. The method comprises in particular the administration of a composition comprising an amount from 10^5 to 10^{12} , in particular from 10^6 to 10^{12} , preferably from 10^8 to 10^{12} bacterial cells

forming colonies CFU per gram of composition (by bacterial cells, is meant a unique bacterial strain in accordance with the invention or a mixture of at least two bacteria, in accordance with the invention). The method comprises in particular the administration of a composition comprising the lactic bacteria(um) in living form. This composition may
5 be a bacterial suspension, which may be frozen and thawed out before use, or further a freeze-dried powder, which may be used as such or after resumption in a suitable carrier. This composition may then comprise a conventional freeze-drying excipient, be an oral administration form (for example, a powder, a capsule, a tablet) optionally in a gastroprotected form allowing crossing the stomach and releasing the bacteria in the
10 intestine, or further a rectal form (for example a suppository).

In one embodiment, the composition used according to the invention in the treatment of malnutrition in mammals, comprises at least one bacterial strain which is not naturally occurring in the species to which the patient to be treated belongs. Thus, this may be a bacterium which is not naturally present in humans or in animals. In this
15 configuration, when there are several different bacteria, it is sufficient that one of them is not naturally present. On the other hand, this bacterium proves to have intestinal tropism in the patient to be treated or in the corresponding species (human or animal according to the invention) and fits the definition of the active strains according to the invention.

20 The invention also concerns a method for screening bacteria capable of promoting juvenile growth in the case of malnutrition in mammals, using a model of an axenic *Drosophila* and/or a model of an axenic mouse.

The method with the *Drosophila* model comprises the following steps:

- a batch of embryos of axenic *Drosophila melanogaster* (e.g. the strain *Drosophila yw*
25 or any other strain called "wild" strain) is available;
- these embryos are distributed into at least 2 groups on a nutritional medium comprising a yeast-deficient nutrient medium for *Drosophila*, one of the groups of embryos is further inoculated with a suspension of the bacterium to be tested, which forms the group of monoxenic individuals; the other group forms the axenic group,
- 30 - the rearing is conducted at a suitable temperature (generally about 25°C),
- at the end of a suitable rearing period (generally about 7 days), *Drosophila* larvae of each group from the initially inoculated or noninoculated embryos (axenic and monoxenic larvae) are recovered, preferably in equal numbers,
- the mean of the length of the larvae of each of the groups is determined and the
35 obtained means are compared;

- the tested bacterial strain is considered as positively responding to the test if the mean of the length of the larvae of the monoxenic group is greater than the mean of the length of the larvae of the axenic group with a p-value less than or equal to 0.001, preferably less than or equal to 0.0001 in the Mann-Whitney statistical test, carried out on the entire of the data set of the sizes of the larvae of both groups.

Preferably, the screening method takes over the features of the *Drosophila* test which was described above.

The method with the mice model comprises the following steps:

- juveniles from two lines of the same mouse strain (typically Balb/c mice) are available, namely an axenic parent mouse line and a monoxenic parent mouse line (associated with the bacterium to be tested),
- they are reared with a protein-deficient and preferably also lipid-deficient nutritional diet,
- at the end of a suitable rearing period, the mean of the values of one or several parameters of each group is determined, these parameters being related to growth (for example increase in weight, skeletal growth for example by measuring the size of the individuals or the size of their femurs), and/or to the serum IGF-1 level,
- the lactic acid bacterium strain is considered as positively responding to the test if the mean value of the measured parameter of the monoxenic group is greater than the mean value of the measured parameter of the axenic group with a p-value less than or equal to 0.05 in Tukey's statistical test.

Preferably, the screening method takes up the features of the mice skeletal growth or the serum IGF-1 level test described above.

The screening method may also be a comparative test with a reference strain, for example the strain WJL, and this test then takes up the features described above for the *Drosophila* or mice tests.

The object of the present invention is also a composition, a pure culture or an isolate comprising the G821 strain (CNCM I-4979), in a living or killed form, a composition comprising this living strain in a preservation or culture medium, a freeze-dried composition comprising this strain and optionally a freeze-drying excipient and/or a preservative, a composition comprising this strain in a killed form in a preservation medium, or killed in a preservation medium, a composition which may be administered to humans or animals, comprising this living or killed strain in a medium or excipient acceptable for oral or rectal administration. The preservation, culture media, the freeze-drying excipients, the preservatives, the media or excipients acceptable for oral or

rectal administration are those described above and those well known to the person skilled in the art.

The invention will now be described in more details by means of the embodiments of the invention taken by way of non-limiting examples and referring to the drawing in which:

5

- Figure 1 is a graph showing the growth rate (in cm/day) of mice from D28 to D56; GF: axenic mice; WJL: mice reared in the presence of the strain *L. plantarum* WJL; NIZO 2877: mice reared in the presence of the strain *L. plantarum* WJL NIZO 2877; * $p < 0.05$; *** $p < 0.001$;

10

- Figure 2 is a graph showing the size of femurs on D56, in GF mice, WJL or NIZO 2877; * $p < 0.05$; *** $p < 0.001$;

- Figure 3 is a graph showing the serum levels (in ng/ml) of male mice on D56, groups GF, WJL or NIZO 2877* $p < 0.05$; *** $p < 0.001$;

15

- Figure 4 is a graph showing the size of larvae of *Drosophila* at 7 days under conditions of under-nourishment: a negative control group (no addition of bacteria on the growth medium after laying), and groups characterised by the addition on the growth medium of the strain *L. plantarum* NIZO 2877, WJL or G821.

Example 1: Functional screening of bacteria on monoxenic *Drosophila*:

20

D-1: axenic adult *Drosophila* are used (*D. melanogaster* yw); they are placed in laying cages including a bottom (of the Petri dish type) on which is placed a conventional nutritional medium; the laying is ensured by maintaining the adult population in the cage for 1 night at 25°C.

25

D0: 6 parts of the nutritional medium are cut out so as to collect 6 times 40 embryos, each of the parts are placed on a deficient nutritional medium contained in a Petri dish, three boxes are inoculated with sterile PBS 1x then the three other ones with a suspension of the bacterium in PBS 1x.

Incubation is performed for 7 days at 25°C.

30

D7: Next, from each box, the larvae which have developed therein (the experiment is operable from 20 larvae per box, we therefore used hereinafter groups of more than 60 individuals) are recovered. The larvae undergo a shock thermal treatment (5 seconds on a hob heated to 100°C), the size of the larvae is then measured.

This protocol was applied to different bacterial strains, and the experiments and results are summarised in the following Table 1:

Table 1.

Sizes of the larvae at D7 (mm)		Statistical analysis				Interpretation of the qualitative test (quantitative level)	
		Comparison/axenic animals		Comparison/ <i>L. plantarum</i> WJL			
	Mean (n>60. mm)	Standard deviation	P-value (1)	Statistical significance	P-value (1)	Statistical significance	
Axenic	2.693	0.497			<0.0001	****	GPS (marked) GPS (intermediate)
<i>Lactobacillus plantarum</i> WJL	3.644 3.398	0.883 0.750	<0.0001 <0.0001	**** ****	0.0001	***	
<i>Lactobacillus plantarum</i> NIZO2877	4.391	0.802	<0.0001	****	0.0721	ns	GPS (marked)
<i>Lactobacillus casei</i> ATCC 393	4.133	0.824	<0.0001	****	>0.9999	ns	GPS (marked)
<i>Lactobacillus fermentum</i> ATCC 9338	2.741 3.287	0.626 0.799	>0.9999 0.1423	ns ns	<0.0001 0.0015	**** **	No effect on growth No effect on growth
<i>Lactobacillus fermentum</i> KLD	3.987	0.524	<0.0001	****	0.4258	ns	GPS (marked)
<i>Lactobacillus fermentum</i> LMG	2.682 3.407	0.482 0.779	>0.9999 <0.0001	ns ****	<0.0001 0.0984	**** ns	No effect on growth GPS (marked)
<i>Lactobacillus paracasei</i> ATCC 25302	2.540	0.599	>0.9999	ns	<0.0001	****	No effect on growth
<i>Lactobacillus paracasei</i> BL23	4.150	0.712	<0.0001	****	0.2966	ns	GPS (marked)
<i>Lactobacillus paracasei</i> Shirota	3.920 4.112	0.572 0.576	<0.0001 <0.0001	**** ****	>0.9999 0.1227	ns ns	GPS (marked) GPS (marked)
<i>Lactobacillus delbrueckii</i> spp.bulgaricus ATCC 11842	4.036	0.488	<0.0001	****	0.5272	ns	GPS (marked)
<i>Lactobacillus casei</i> L919							

[illegible]

(1) Statistical test used: Mann-Whitney test.

Indications on the validation criteria of the “larval growth” phenotype test

The strain should at least fulfil criterion 1 in order to be validated and the extent of its activity may be described according to the criterion 1+2.

Qualitative criterion 1 = growth promoting strain relatively to the axenic condition:

A statistical test is applied to the whole of the values of length of the individuals associated relative to the values of the axenic individuals (at least 60 larvae per condition). A p-value less than 0.001 (Mann-Whitney test) qualifies the strain as “growth promoting”. In the present case, the following strains are qualified as growth-promoting:

Lactobacillus plantarum WJL
Lactobacillus plantarum NIZO2877
Lactobacillus casei ATCC 393
Lactobacillus fermentum ATCC 9338
Lactobacillus paracasei ATCC 25302
Lactobacillus paracasei Shirota
Lactobacillus casei L919
Lactobacillus rhamnosus L900
Lactobacillus rhamnosus L908
Lactobacillus rhamnosus GG

The following strains do not have any growth-promoting effect:

Lactobacillus fermentum KLD
Lactobacillus fermentum LMG
Lactobacillus paracasei BL23
Lactobacillus delbrueckii spp.bulgaricus ATCC 11842

Quantitative criterion 2 = extent of the growth promoting effect (with criterion 1 having been validated beforehand):

A statistical test is applied to the whole of the values of length of the individuals associated with the tested strain(s) relative to the values of the mono-associated individuals with the strain *L.plantarum* WJL (at least 60 larvae per condition).

Three scenarios occur:

The p-value of the statistical test is greater than 0.001 (Mann-Whitney test), the strain will be qualified as having a “marked” effect on the larval growth (effect similar to the strain *L.plantarum* WJL).

In the present case, the following strains have a marked effect:

Lactobacillus casei ATCC 393
Lactobacillus fermentum ATCC 9338

Lactobacillus paracasei ATCC 25302

Lactobacillus paracasei Shirota

Lactobacillus casei L919

Lactobacillus rhamnosus L900

5 *Lactobacillus rhamnosus* L908

Lactobacillus rhamnosus GG

Lactobacillus plantarum WJL

10 The p-value of the statistical test is less than 0.001 (Mann-Whitney test), the strain will be described as having an “intermediate” effect on larval growth if the mean size of the individuals is less than that of the individuals associated with *L.plantarum* WJL (case of the strain *L.plantarum* NIZO 2877 in the shown example). The strain will be qualified as having a “strong” effect if the mean size of the individuals is greater than that of the individuals associated with *L.plantarum* WJL (a non-identified case in the present example).

15

Analysis of the “larval growth” phenotype test example:

- 14 *Lactobacillus* strains were tested (Table 1) in order to determine their growth promoting potential by means of the “larval growth” test in *Drosophila melanogaster*.
 - These strains are available from ATCC, at the Pasteur Institute of Paris, at the Pasteur Institute of Lille or published in the scientific literature and available from the referee researchers of the mentioned publications (Table 2).
- 20

Table 2:

	Public collection and/or publication with sequence of the genome
<i>Lactobacillus plantarum</i> WJL	Kim et al., Genome Announc. November 21, 2013, 1(6). Pii : e00937-13
<i>Lactobacillus plantarum</i> NIZO2877	NIZO (2877)
<i>Lactobacillus casei</i> ATCC 393	ATCC (393)
<i>Lactobacillus fermentum</i> ATCC 9338	ATCC (9338)
<i>Lactobacillus fermentum</i> KLD	Pasteur Institute of Lille (A5.20)
<i>Lactobacillus fermentum</i> LMG	Pasteur Institute of Lille (A5.16)
<i>Lactobacillus paracasei</i> ATCC 25302	ATCC (25302)
<i>Lactobacillus paracasei</i> BL23	Pasteur Institute of Lille (A3.5)
<i>Lactobacillus paracasei</i> Shiota	ATCC (11842) and van de Guchte M, et al., Proc.Natl Acad Sci USA June 13, 2006
<i>Lactobacillus delbrueckii</i> spp.bulgaricus	Koryszewska-Baginska A et al., Genome Announc, September 26, 2013
<i>Lactobacillus casei</i> L919	Aleksandrak-Piekarczyk T et al. Genome Announc, August 15, 2013
<i>Lactobacillus rhamnosus</i> L900	Koryszewska-Baginska A et al. Genome Announc, February 20, 2014
<i>Lactobacillus rhamnosus</i> L908	ATCC (53103) and Kankainen M et al., Proc Natl Acad Sci USA, October 6 2009
<i>Lactobacillus rhamnosus</i> GG	

- The size of a minimum of 60 individuals per condition is studied on day 7 after association with the bacterial strain to be tested, and then rearing of the juvenile individuals in a yeast-deficient nutritional medium. Table 1 shows the mean and the standard deviation of these sets of data.
- 5 – The statistical analysis of these results allows qualifying according to criterion 1 of the “larval growth test”, 10 strains of different species of *Lactobacilli*. This selection brings to light a strictly strain-specific functional effect and is not necessarily related to a given species of *Lactobacillus*.
- 10 – The statistical analysis of the results allows according to criterion 2, qualifying as “intermediate” the effect of the strain *L. plantarum* NIZO 2877 while the 9 other promoting growth strains have “marked” effects.

Example 2: Description of a strain with a strong effect on the *Drosophila* model

The *Drosophila* test of Example 1 is reproduced with 3 bacterial strains:

- 15 - *Lactobacillus plantarum* WJL
 - *Lactobacillus plantarum* NIZO2877
 - *Lactobacillus plantarum* G821

The analysis of the results (see Figure 4) shows the following effects:

- 20 Axenic versus NIZO 2877 $p < 0.001$ (***)
 NIZO 2877 versus WJL $p < 0.0001$ (****)
 G821 versus WJL $p < 0.0001$ (****)
 Axenic versus WJL or G821 $p < 0.0001$ (****)

25 Conclusion:

- *Lactobacillus plantarum* WJL: marked effect
- *Lactobacillus plantarum* NIZO2877: intermediate effect
- *Lactobacillus plantarum* G821: strong effect.

30 Example 3: Functional screening of bacteria on mice – phenotype analysis:

Study in mice of the juvenile growth promoting effect of two strains with “intermediate” and “marked” effect (*L. plantarum* NIZO2877 and *L. plantarum* WJL, respectively) identified by means of the “larval growth” test of Example 1.

- 35 The male progeny (a minimum of 15 individuals) of three groups of individuals from the same axenic mouse colony was studied, the first group consists in axenic juveniles (group GF), the second one in juveniles issued from mono-associated parents with the strain

L. plantarum WJL (group WJL), and the third one in juveniles from mono-associated parents with the strain *L. plantarum* NIZO2877 (group NIZO 2877). The parents and juveniles are reared in a conventional nutritional medium until weaning of the juveniles (J21), and then the weaned juveniles are reared in a protein-deficient and lipid-deficient (8% and 2.5% respectively) nutritional medium up to D56.

Conventional nutritional medium: 23% proteins and 5% lipids.

Deficient nutritional medium: 8% proteins and 2.5% of lipids.

Three parameters illustrating the juvenile growth of these individuals were studied: (1) the increase in size for a period of 28 days following weaning (from D28 to D56), (2) the size of the femurs of a batch of individuals (a minimum of 9 individuals) representative of the tested population at D56 and finally (3) the serum level at D56 of the IGF-1 growth factor in at least 8 individuals.

The statistical analyses were carried out using the Tukey's test by using the GraphPad software (GraphPad Prism 5.04, San Diego, USA); p-values < 0.05 are considered as significant.

(1) Increase in size:

The mice were anesthetised by brief exposure to isoflurane to allow measuring the size of the mice (from the snout to the base of the tail) on D28 and D56.

Table 3: growth rate (size increase per day in cm from D28 to D56) – Nb = number of mice

GF			WJL			NIZO 2877		
Mean	Standard deviation	Nb	Mean	Standard deviation	Nb	Mean	Standard deviation	Nb
0.007321	0.009878	15	0.031114	0.009376	17	0.016429	0.011071	25

These data are collected in Figure 1.

(2) Size of the femurs:

The mice were sacrificed on D56, a femur was sampled, released from the muscle and its length was measured with the Vernier calliper.

Table 4: Length of the femurs in mm on D56 in males

GF	WJL	NIZO 2877
10.2	11.2	10.9
10.2	11.6	11
10.5	11.9	10.5
9.9	11.6	11.2
10.4	12	11
10	11.4	11

10.2	12	11
10.2	11.7	11.2
9.8	12	11
	11.9	
	11,6	
	11	

These data are collected in Figure 2.

(3) IGF titres in -1 in the serum:

The IGF-1 titre is measured on the serum obtained from the blood of the sacrificed mice at D56. The measurement is performed on diluted serum (1:25) by using the ELISA Ready-Set-Go kit (eBioscience, USA), by following the instructions of the manufacturer.

Table 5: Serum IGF-1 titre in pg/ml (dual measurement from ELISA plates)

GF		WJL		NIZO 2877	
4526.75	4343.75	8823.25	8606.5	7291.25	6998.75
2825.5	2614.75	9258.5	9122.25	5128	5102
2667.5	2773	8471.25	8660.5	4343.75	4396
3455.25	3219.5	7025.25	7078.25	5232.75	5180.5
7131.5	6839.5	11063	9557.3	8471.25	8282.5
2164.2	2057.55	15125	14409	4866.5	4761.75
3140.75	3035.75	10023	9372	6363.25	6152.25
2509	2456.15	11735	12026	7211.25	6680.5
2588.25	2614.75			4213.25	4161
4432.5	4601.3			4770.5	4517
4013.3	3930			4940.3	3846.5
3597.3	3846.5				

These data are collected in Figure 3.

The results illustrate a “marked” effect of the strain *L. plantarum* WJL on the growth rate (p-value <0.001 relative to the axenic condition) and an “intermediate” effect of the strain *L. plantarum* NIZO2877 (p-value <0.05 relative to the axenic condition and p-value <0.001 relative to the condition *L. plantarum* WJL). These effects are confirmed with the “IGF-1 level” parameter (same ranges of p values of the different tests), but reinforced with the “femur size” parameter (p-value <0.001 between the axenic conditions and *L. plantarum* NIZO 2877).

All these results demonstrates by scientific proof, the juvenile growth promoting effect of some strains of *Lactobacilles* also positively responding to the “larval growth” test.

The person skilled in the art may refer to the deposits of commercial strains to which reference is made herein.

P a t e n t k r a v

1. Sammensætning, omfattende mindst én *Lactobacillus*-stamme med intestinal tropisme, der er udvalgt fra arterne *Lactobacillus plantarum* eller *Lactobacillus fermentum*, til anvendelse til fremme af ungdomsvækst ved behandling af fejlnæring hos pattedyr, hvor *Lactobacillus*-stammen reagerer positivt på mindst en af følgende tests A), B), C) og D):

Test A)

- et parti embryoner fra akseniske *Drosophila melanogaster*-forældre er tilgængelige;
- J1: disse embryoner fordeles i mindst 2 grupper (40 embryoner, udført i tre eksemplarer, dvs. 120 embryoner pr. gruppe) på et ernæringsmedium, der omfatter majsmeal, agar og vand, der mangler gær, en af embryogrupperne er yderligere podet med en suspension på ca. 10^8 CFU af bakterien, der skal testes i en saltopløsningsbuffer, hvilket danner den monoxeniske gruppe, hvor den anden gruppe er den axeniske gruppe, opdræt af begge grupper udføres ved ca. 25°C frem til J7;
- J7: mindst 60 *Drosophila*-larver fra hver gruppe genvindes og udsættes for temperaturchok;
- gennemsnittet af længden af larverne i hver gruppe bestemmes, og de opnåede gennemsnit sammenlignes;
- den testede bakteriestamme anses for at reagere positivt på testen, hvis gennemsnittet af larvernes længde af den monoxeniske gruppe er større end gennemsnittet af larvernes længde i den axeniske gruppe med en p-værdi, der er mindre end eller lig til 0,001, fortrinsvis mindre end eller lig med 0,0001 i den statistiske Mann-Whitney-test, der er udført på hele datasættet af størrelserne på larverne i begge grupper

Test B)

- fra den samme muselinje etableres en linje af axeniske forældremus og en linje af monoxeniske forældremus (forbundet med bakterien, der skal testes),

og der produceres unger, der opdrættes med forældrene i et konventionelt ernæringsmedium, indtil de er fravænnet (J21)

5 - J21: til rådighed er 8 fravænnede unger, der stammer fra hver af disse to linjer, der danner den monoxeniske gruppe og den axeniske gruppe, de opdrættes på en ernæringsmæssig diæt, der mangler protein (8%) og lipider (2,5%);

- J56: middelstørrelsen af musene for hver betragtet gruppe bestemmes ved at måle fra enden af snuden til bunden af halen på hvert individ, eller lårbenene for individerne i hver gruppe udtages og måles;

10 - stammen af mælkesyrebakterier anses for at reagere positivt på testen, hvis middelstørrelsen af individerne eller lårbenene af den monoxeniske gruppe er større end middelstørrelsen for individerne eller lårbenene af den axeniske gruppe, med en p-værdi, der er mindre mere end eller lig med 0,05 i den statistiske Tukey-test;

15 Test C)

- fra den samme axeniske muselinje etableres en linje af axeniske forældremus og en linje af monoxeniske forældremus (forbundet med bakterien, der skal testes), og der produceres unger, der opdrættes med forældrene i et konventionelt ernæringsmedium, indtil de er fravænnet (J21)

20 - J21: til rådighed er 8 fravænnede unger, der stammer fra hver af disse to linjer, der danner den monoxeniske gruppe og den axeniske gruppe, de opdrættes på en ernæringsmæssig diæt, der mangler protein (8%) og lipider (2,5%);

25 - J56: der udtages blod fra ungerne af hver gruppe, og det gennemsnitlige serum-IGF-1-niveau bestemmes for hver gruppe;

- stammen af mælkesyrebakterier anses for at reagere positivt på testen, hvis det gennemsnitlige serum-IGF-1-niveau af den monoxeniske gruppe er større end den gennemsnitlige serumandel af den axeniske gruppe, med en p-værdi, der er mindre mere end eller lig med 0,05 i den statistiske Tukey-test

30 Test D)

- et parti embryoner fra akseniske *Drosophila melanogaster*-forældre er tilgængelige;
- J1: disse embryoner fordeles i mindst 2 grupper (40 embryoner, udført i tre eksemplarer, dvs. 120 embryoner pr. gruppe) på et ernæringsmedium, der omfatter majsmeal, agar og vand, der mangler gær, en af embryogrupperne er yderligere podet med en suspension på ca. 10^8 CFU af bakterien i en saltopløsningsbuffer, hvilket danner den monoxeniske testgruppe, hvor den anden gruppe podes med en suspension på ca. 10^8 CFU af referencebakterien *L. plantarum* WJL eller *L. fermentum* ATCC 9338, i en saltvandsbuffer, der danner den monoxeniske referencegruppe, opdræt af begge grupper udføres ved ca. 25°C frem til J7;
- J7: mindst 60 *Drosophila*-larver fra hver gruppe genvindes og udsættes for temperaturchok;
- gennemsnittet af længden af larverne i hver gruppe bestemmes, og de opnåede gennemsnit sammenlignes;
- bakteriestammen har i forhold til referencebakterien en stærk effekt med et gennemsnit af længden af larverne i den monoxeniske testgruppe med denne bakteriestamme, der er betydeligt højere end middelværdien for den monoxeniske gruppe med referencestammen, med en p-værdi, der er mindre end eller lig med 0,001, fortrinsvis til 0,0001 i den statistiske Mann-Whitney-test, der er udført på hele datasættet af størrelserne på larverne i begge grupper.

2. Sammensætning til anvendelse ifølge krav 1, hvor *Lactobacillus*-stammen er valgt blandt *L. plantarum* WJL, *L. plantarum* G821, og *L. fermentum* ATCC9338.

3. Sammensætning til anvendelse ifølge krav 1, som i test A) omfatter en bakteriestamme, der i forhold til stammen *L. plantarum* WJL i den samme *Drosophila*-test, har en stærk effekt med et gennemsnit af længden af larverne i den monoxeniske testgruppe med denne bakteriestamme, der er betydeligt højere

end middelværdien for den monoxeniske gruppe med stammen WJL, når p-værdien er mindre end eller lig med 0,001, fortrinsvis til 0,0001 i den statistiske Mann-Whitney-test, der er udført på hele datasættet af størrelserne på larverne i begge grupper.

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4. Sammensætning til anvendelse ifølge krav 1, som i test B) omfatter en bakteriestamme, der i forhold til stammen *L. plantarum* WJL i den samme muse-test, har en stærk effekt med et gennemsnit af musenes størrelse eller deres lårben i den monoxeniske testgruppe med denne bakteriestamme, der er betydeligt større end gennemsnittet for den monoxeniske gruppe med stammen WJL, når p-værdien er mindre end eller lig med 0,05 i den statistiske Tukey-test.

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5. Sammensætning til anvendelse ifølge krav 1, som i test C) omfatter en bakteriestamme, der i forhold til stammen *L. plantarum* WJL i den samme muse-test, har en stærk effekt med et gennemsnitligt serum-IGF-1-niveau af i den monoxeniske testgruppe med denne bakteriestamme, der er betydeligt større end gennemsnittet for den monoxeniske gruppe med stammen WJL, når p-værdien er mindre end eller lig med 0,05 i den statistiske Tukey-test.

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6. Sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 5, hvor fejlnæringen hos pattedyr er **kendetegnet ved** en proteinmangel.

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7. Sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, hvor fejlnæringen hos pattedyr er **kendetegnet ved** et serum-IGF-1-niveau, der er under normalen.

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8. Sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, hvor bakterien er en *Lactobacillus plantarum*.

9. Sammensætning til anvendelse ifølge krav 8, hvor bakterien er en *Lactobacillus plantarum* G821, der er deponeret i Collection Nationale de Culture de Microorganismes (Institut Pasteur) under registreringsnummeret CNCM I-4979.

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10. Sammensætning til anvendelse ifølge et hvilket som helst af de foregående krav, der indeholder fra 10^5 til 10^{12} CFU *Lactobacillus*, der reagerer positivt på testen, pr. gram sammensætning.

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11. Fremgangsmåde til screening af bakterier, der er i stand til at fremme ungdomsvækst i tilfælde af fejlemæring af pattedyr, især mennesker, husdyr, kæledyr og sportsdyr, hvor bakteriestammen udsættes for følgende test:

- et parti embryoner fra akseniske *Drosophila melanogaster*-forældre er tilgængelige;

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- disse embryoner placeres i mindst 2 grupper på et ernæringsmedium, der omfatter et gærfattigt næringsmedium til *Drosophila*, en af embryogrupperne inokuleres yderligere med en suspension af den bakterie, der skal testes, hvilket danner gruppen af monoxeniske individer; den anden gruppe danner den axeniske gruppe,

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- opdræt foregår ved en passende temperatur,
- ved afslutningen af en passende opdrætsperiode genvindes *Drosophila*-larver fra hver gruppe (akseniske og monoxeniske larver), fortrinsvis i lige store mængder,

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- gennemsnittet af længden af larverne i hver gruppe bestemmes, og de opnåede gennemsnit sammenlignes;
- den testede bakteriestamme anses for at reagere positivt på testen, hvis gennemsnittet af larvernes længde af den monoxeniske gruppe er større end gennemsnittet af larvernes længde i den axeniske gruppe med en p-værdi, der er mindre end eller lig til 0,001, fortrinsvis mindre end eller lig med 0,0001 i Mann-

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- Whitney-testen, der er udført på hele datasættet.

12. Fremgangsmåde til screening af bakterier, der er i stand til at fremme ungdomsvækst i tilfælde af fejlmæring, hvor bakteriestammen udsættes for følgende test:

- 5 - unge er tilgængelige fra to linjer, der stammer fra samme musestamme, nemlig en axenisk muselinje og en monoxenisk muselinje,
- de er opdrættet med en ernæringsdiæt med proteinmangel og fortrinsvis også lipidfattig;
- ved afslutningen af en passende opdrætningsperiode bestemmes middelværdien af værdierne for en eller flere parametre i hver gruppe, hvor disse
- 10 parametre er relateret til serum-IGF-1-niveauet eller til væksten,
- stammen af mælkesyrebakterier anses for at reagere positivt på testen, hvis det gennemsnitlige IGF-1-niveau af den monoxeniske gruppe er større end det gennemsnitlige niveau af den axeniske gruppe, med en p-værdi, der er mindre
- 15 mere end eller lig med 0,05 i den statistiske Tukey-test.

13. Sammensætning, en ren kultur eller et isolat, der omfatter bakterien *Lactobacillus plantarum* G821, der er deponeret i Collection Nationale de Culture de Microorganismes (Institut Pasteur) under registreringsnummeret CNCM I-4979.

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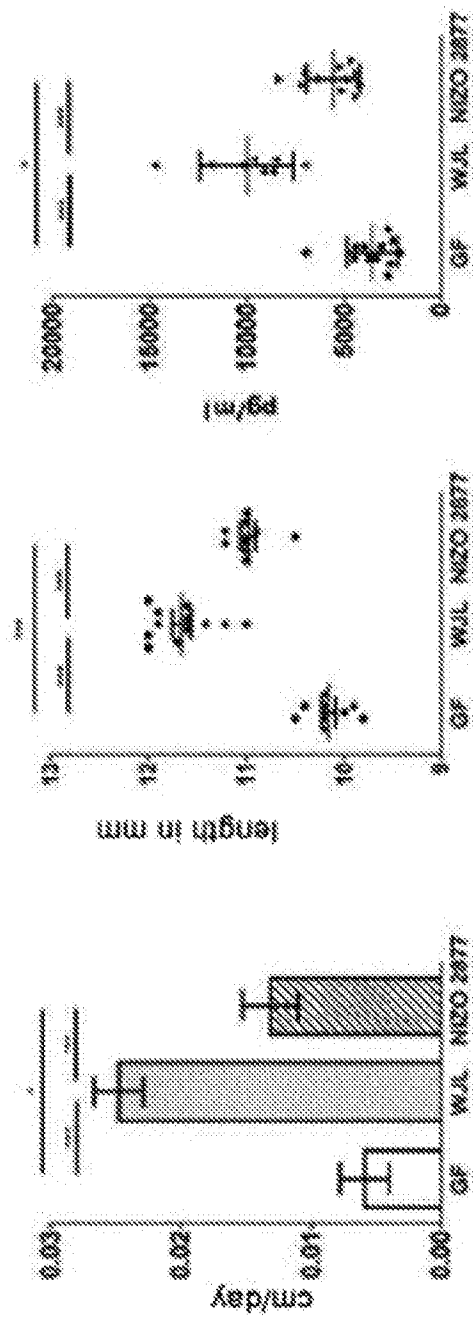


Fig.1

Fig.2

Fig.3

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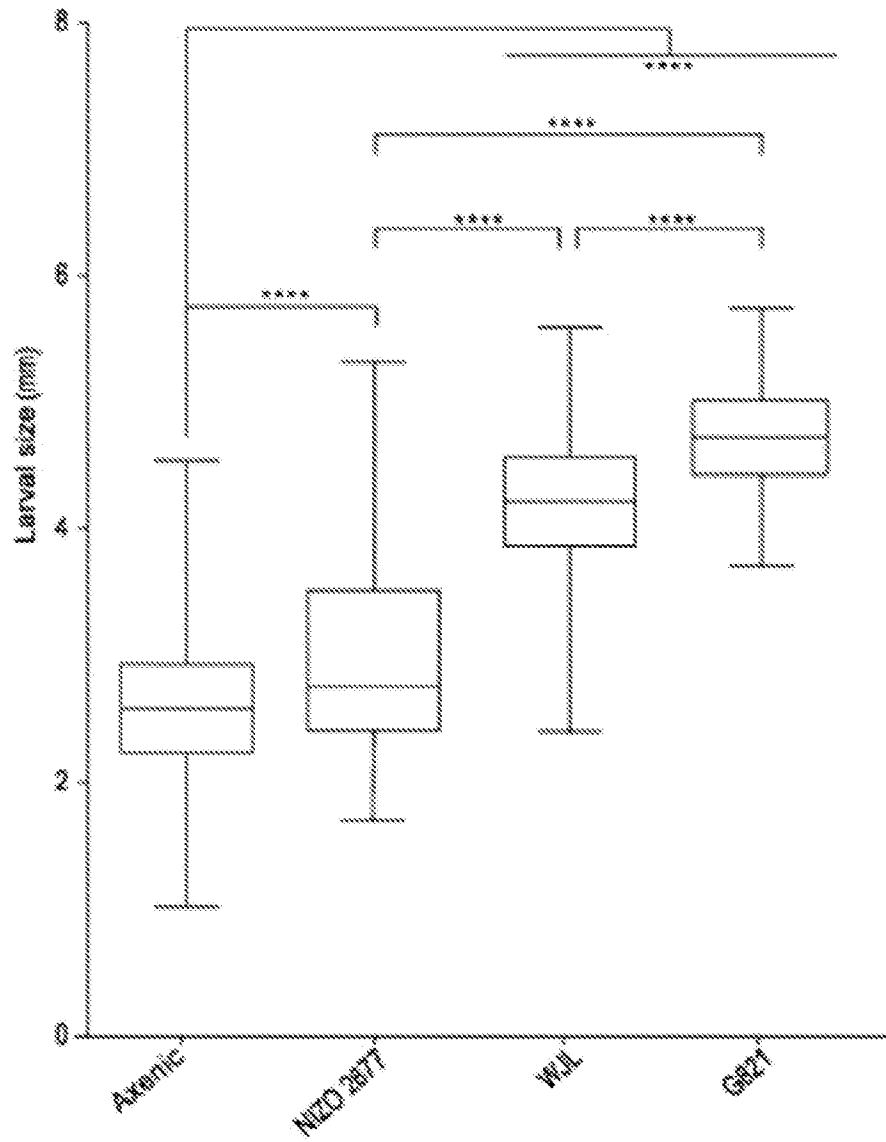


Fig.4