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(54) Title: PROBIOTIC BACTERIA BASED COMPOSITION AND USE THEREOF IN THE PREVENTION AND/OR TREATMENT OF RESPIRATORY PATHOLOGIES AND/OR INFECTIONS AND IN THE IMPROVEMENT OF THE INTESTINAL FUNCTIONALITY

(57) Abstract: The object of the present invention is a probiotic bacteria based composition and use thereof in the prevention and/or treatment of respiratory pathologies and/or infections and in the contemporaneous improvement of the intestinal functionality, which can be compromised by therapeutic treatments adopted for re-solving said pathological conditions. Besides that, aim of this invention is a probiotic bacteria-based composition comprising moreover a suitable quantity of at least a substance or nourishment having prebiotic properties and eventually, of suitable pharmacologically active substances.



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PROBIOTIC BACTERIA BASED COMPOSITION AND USE THEREOF
IN THE PREVENTION AND/OR TREATMENT OF RESPIRATORY PA-
THOLOGIES AND/OR INFECTIONS AND IN THE IMPROVEMENT OF
THE INTESTINAL FUNCTIONALITY

* * * * *

DESCRIPTION

The present invention relates to probiotic bacteria based compositions, if necessary integrated with prebiotic substances, and the use thereof in the prevention and/or treatment of respiratory pathologies and/or infections and in the contemporaneous improvement of the intestinal functionality, which may result compromised from therapeutic treatments adopted for resolving said pathological conditions.

In the last fifty years, the employ in the food field of probiotic bacteria has gained an always increasing importance.

For "probiotic" it is understood to mean those living species-specific microorganisms which, when swallowed or applied in a sufficient number, are able to induce in the consumer specific functional and beneficial effects on the state of health of the host.

If the action of the probiotic microorganism plays a pharmacologically active role towards pathological forms concerning the host, the probiotic microorganism

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can be defined by the term "biotherapeutic agent", to testify the potential ability of supplying a valid help to the medical therapy.

Therefore, considering that the beneficial properties of the probiotics can be both of a general systemic order and aimed to solve specific disorders or diseases, their use concerns various applicative fields, from the food industry to the pharmaceutical one.

In the pharmaceutical field, the probiotic bacteria are usually employed, for example, in the prevention and treatment of intestinal pathologies of a different origin and nature.

The possible beneficial action of the probiotic bacteria is also object of study, for example in patients suffering from diabetes mellitus of type 2, chronic autoimmune and inflammatory pathologies, tumor pathologies, high serum levels of cholesterol.

However, the use of probiotic bacteria in the prevention and/or therapeutic treatment of the respiratory pathologies is not known.

In particular, the use of probiotic bacteria, in association, or not, with opportune substances having prebiotic properties for the prevention and/or treatment of respiratory pathologies and/or infections and, at the same time, for the improvement and/or the regu-

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larization of the intestinal functionality, which can result compromised (indeed, very often it results compromised) by therapeutic treatments adopted for resolving said pathological conditions, is not known.

Usually, the respiratory pathologies and/or infections are treated by using the administration, sometimes also massive and for prolonged times, of antibiotics and/or antiinflammatories.

Unfortunately, the side effects caused by the use of these drugs are often troublesome, harmful and weakening for the organism.

The object of the present invention is to prevent and/or therapeutically treat respiratory pathologies and/or infections of a various origin and nature, without causing undesired side effects, such as those induced by the traditional treatments with antibiotics and/or antiinflammatories.

Another of the objects of the present invention is to prevent and/or therapeutically treat respiratory pathologies and/or infections of a various origin and nature, by simultaneously improving and/o regularizing the intestinal functionality of the organism, often compromised by said pathological conditions.

These and other objects, which will better result from the following detailed description, have been attained

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by the Applicant, which has unexpectedly found that a composition including an opportune mixture of probiotic bacteria is able to give an adequate response to the problems above-pointed out.

An object of the present invention is therefore the use of a composition including a mixture of probiotic bacteria for the preparation of a medicament for the prevention and/or the therapeutic treatment of respiratory pathologies and/or infections, as summarized in the appended independent claim.

Another object of the present invention is a composition for the use above-mentioned, including a mixture of probiotic bacteria, belonging to the genus *Lactobacillus* and/or genus *Bifidobacterium*, whose features are summarized in the appended independent claim.

A kit for the co-ordinated administration of said composition in combination with one or more pharmacologically active substances, as summarized in the appended independent claim, forms then a further object of the present invention.

Preferred embodiments of the present invention are summarized in the appended dependent claims.

In a preferred embodiment, the composition of the present invention is used for the preparation of a medicament for the prevention and/or the therapeutic

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treatment of respiratory pathologies and/or infections.

In another preferred embodiment, the composition of the present invention is used for the preparation of a medicament for the prevention and/or the therapeutic treatment of respiratory pathologies and/or infections, with a simultaneous improvement and/or regularization of the intestinal functionality of the organism.

In another preferred embodiment, the composition of the present invention is used for the preparation of an influenza vaccine.

In a further preferred embodiment, the composition of the present invention includes a mixture of three probiotic bacterial species, belonging to the genus *Lactobacillus* and the genus *Bifidobacterium*.

In a further preferred embodiment, the composition of the present invention additionally includes at least a substance having prebiotic properties.

By the term "prebiotic", are generally shown substances or components of the diet (such as, for example, fibres), neither digestible nor absorbable by the organism, which, when arrived integral in the colon, selectively stimulate the development and the activity of the microbial groups beneficial for the health of

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the individual.

The association of probiotics with prebiotic substances or foods gives rise to compositions generally shown by the term "symbiotic".

In another preferred embodiment, said composition additionally includes at least another probiotic bacterium.

In an additional preferred embodiment, said composition further includes at least a pharmacologically active substance.

The compositions of the present invention proved useful preferably for the prevention and/or treatment of the following pathologies:

- influenza like syndromes, often characterized by fever and affections at the expense of the respiratory system (usually shown in the art with the ILI abbreviation, that is Influenza Like Illness);
- bronchitic pathologies of a different nature (including those of a chronic type);
- pathologies concerning the high respiratory tree, such as for example, laryngitis and tracheitis (generally shown in the art by the URTI abbreviation, that is Upper Respiratory Tract Infections);
- common cold;
- cough.

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The compositions of the present invention include at least a probiotic bacterial species.

Preferably, said compositions include a mixture of multiple bacterial probiotic species.

More preferably, said probiotic bacterial species are opportunely selected among those belonging to the genus *Lactobacillus* and/or genus *Bifidobacterium*.

In a particularly preferred embodiment, said compositions include a mixture consisting of the following three bacterial species: *Bifidobacterium lactis*, *Lactobacillus casei* subsp. *rhamnosus*, *Lactobacillus plantarum*.

Advantageously, particularly preferred bacterial strains belonging to said mixture of three bacterial species are resulted those selected from:

- *Bifidobacterium lactis*, ID n. LMG P-21384 (deposited to the Belgian Coordinated Collections of Microorganisms - BCCM LMG Collection, on January 31st 2002);
- *Lactobacillus casei* subsp. *rhamnosus*, ID n. DSM 16605 (deposited to the DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany, on July 20th 2004);
- *Lactobacillus plantarum*, ID n. LMG P-21021 (deposited to the Belgian Coordinated Collections of Microorganisms - BCCM LMG Collection, on October 16th

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2002);

- *Lactobacillus plantarum*, ID n. LMG P-21020 (deposited to the Belgian Coordinated Collections of Microorganisms - BCCM LMG Collection, on October 16th 2002);

- *Lactobacillus plantarum*, ID n. LMG P-21022 (deposited to the Belgian Coordinated Collections of Microorganisms - BCCM LMG Collection, on October 16th 2002);

- *Lactobacillus plantarum*, ID n. LMG P-21023 (deposited to the Belgian Coordinated Collections of Microorganisms - BCCM LMG Collection, on October 16th 2002).

In another particularly preferred embodiment, the compositions of the present invention further include at least a substance having prebiotic properties.

Said prebiotic preferably includes carbohydrates which are not digested and absorbed by the organism.

Said carbohydrates are preferably selected from: fructo-oligosaccharides (or FOS), short-chain fructo-oligosaccharides, inulin, isomalt-oligosaccharides, pectins, galacto-oligosaccharides (or GOS), arabinogalactan, xylo-oligosaccharides (or XOS), chitosan-oligosaccharides (or COS), glucanmannan, beta-glucans, Konjac, guar, arabic, xanthan gums, modified and re-

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sistant starches, polydextrose, D-tagatose.

Particularly preferred prebiotics are the short-chain fructo-oligosaccharides (for simplicity shown hereinbelow as FOSs-c.c.); said FOSs-c.c. are not digestable glucides, generally obtained by the conversion of the beet sugar and including a saccharose molecule to which three glucose molecules are bonded.

In another particularly preferred embodiment, the compositions of the present invention further include, in addition to the characterizing mixture, consisting of the three bacterial species above-defined, at least another probiotic bacterium.

Preferably, said additional probiotic is selected among the following bacterial species:

Bifidobacterium adolescentis, *Bifidobacterium angulatum*, *Bifidobacterium animalis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium catenulatum*, *Bifidobacterium denticolens*, *Bifidobacterium dentium*, *Bifidobacterium gallicum*, *Bifidobacterium infantis*, *Bifidobacterium inopinatum*, *Bifidobacterium longum*, *Bifidobacterium pseudocatenulatum*, *Enterococcus faecium*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus casei subsp. paracasei*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus fermentum*, *Lactobacillus GG*, *Lactobacillus pentosus*, *Lac-*

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tobacillus salivarius, *Saccharomyces boulardi*, *Streptococcus thermophilus*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *diacetylactis*.

Additional particularly preferred bacterial strains belonging to said probiotic bacterial species are those selected from:

- *Lactobacillus acidophilus*, ID n. LMG P-21381 (deposited to the Belgian Coordinated Collections of Microorganisms - BCCM LMG Collection, on January 31st 2002);
- *Lactobacillus casei* subsp. *paracasei*, ID n. LMG P-21380 (deposited to the Belgian Coordinated Collections of Microorganisms - BCCM LMG Collection, on January 31st 2002);
- *Lactobacillus pentosus*, ID n. LMG P-21019 (deposited to the Belgian Coordinated Collections of Microorganisms - BCCM LMG Collection, on October 16th 2002);
- *Lactobacillus delbrueckii* subsp. *bulgaricus*, ID n. DSM 16607 (deposited to the DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany, on July 20th 2004);
- *Lactobacillus delbrueckii* subsp. *bulgaricus*, ID n. DSM 16606 (deposited to the DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany, on July 20th 2004).

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In another particularly preferred embodiment, the compositions of the present invention further include at least a pharmacologically active substance, so as to be able to perform a combined action to that exerted by said active substances.

Advantageously, such associations are revealed synergic, accordingly allowing the use of relatively low doses of pharmaceutical active substance, with a considerable decrease of the possible side effects induced by the administration of the drug alone.

Preferred pharmacologically active substances are, for example, selected from:

antibiotics, antiinflammatories, immunomodulating, mucolytics, antispasmodics, vitamins.

The composition of the invention are preferably formulated in admixture with appropriate excipients, such as carriers, lubricants, dispersers, flavourings, sweeteners, stabilizers, preservatives, antioxidants, additives, such as amino acids, vitamins, enzymes, generally used in the formulation and the pharmaceutical art.

By mere way of absolutely non limiting example, among the particularly preferred excipients and additives there may be mentioned starch, tween, fragrances, such as those of mandarin, grapefruit, strawberry, bil-

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berry, all fruits, saccharose, glucose, acesulfame, aspartame, ascorbic acid, parabens, glutamine, arginine, superoxide dismutase, glutathione.

Particularly preferred compositions of the present invention are those for oral administration.

Typical preferred embodiments are, for example, capsules, beads, solutions or suspensions ready to drink, powders or granulates sachets (to be suspended or dissolved in water or non-carbonated and non-alcoholic beverages at the time of use) or similar forms, tablets, effervescent formulations.

The compositions of the present invention can also be formulated in a coated, lacquered, encapsulated or microencapsulated form, so as to be gastroresistant.

Said composition can also be formulated in a controlled-release form, so as to selectively release the active substances in the intestinal tract, particularly in the colon.

Among the preferred embodiments of the present invention, there may be mentioned those formulations wherein the preferred bacterial strains of the invention are preferably used in a freeze-dried form.

The freeze-drying of said strains, alone or in admixture with opportune excipients, is carried out by using techniques and equipments generally employed in

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the freeze-drying processes of pharmaceutical and/or food compositions.

The compositions of the present invention are prepared in a traditional way by using, depending on the type of formulation that one wishes to prepare, preparative techniques known to the skilled in the pharmaceutical sector.

By way of absolutely non limiting example, a granular formulation, to be suspended or dissolved in water at the time of use, will be prepared by intimately mixing the components of the composition (active substances, co-adjuvants, excipients), reducing them to the desired granulometry and moisture degree, before packing them in single dose sealed sachets.

In turn, a controlled-release composition will be, for example, prepared by microencapsulating or microcoating the microgranulated mixture of the substances constituting the formulation with opportune mixtures of biocompatible polymers (such as, for example, Eudragit of different type and structure) resistant to the gastric juices of the stomach and able to release said components after a proper residence time in the gastrointestinal tract, or at pH values typical of the colon.

The microencapsulated mixture thus obtained will be,

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for example, used for the preparation of tablets, capsules or beads, depending on the selected commercial kind of presentation.

As for the dosage, the bacterial species constituting the mixture of probiotic bacteria characterizing the present invention are present in a mutual weight ratio between 0.1:10 to 10:1, preferably in a mutual weight ratio between 0.5:1 to 2:1, more preferably in a mutual weight ratio of about 1:1.

In the particularly preferred case wherein said mixture of probiotic bacteria consists of the three bacterial species *Bifidobacterium lactis*, *Lactobacillus casei* subsp. *Rhamnosus*, *Lactobacillus plantarum*, said bacterial species are preferably present in a weight ratio of 1:1:1.

The additional probiotic, if any, is present in a weight ratio between 0:1 to 100:1, with respect to the total quantitative of the bacterial species of said mixture; preferably, in a weight ratio between 2:1 to 15:1; more preferably, from 5:1 to 10:1.

The additional probiotic, if any, is present in a weight ratio between 0:1 to 10:1, with respect to the total quantity of the bacterial species of said mixture; preferably, in a weight ratio from 0.5:1 to 3:1. Each single bacterial species of said mixture of pro-

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biotic bacteria of the present invention is present in a concentration between $1 \cdot 10^6$ UFC/dose to $1 \cdot 10^{12}$ UFC/dose; preferably, from $1 \cdot 10^9$ UFC/dose to $1 \cdot 10^{11}$ UFC/dose.

In a particularly preferred embodiment of the invention, a granulate for oral use, packed in single dose sachets to be dissolved in water before the intake is prepared.

By way of absolutely non limiting example, some preferred compositions of the invention are given hereinbelow.

Composition A

In a single dose sachet, 5 g of granulate for oral use are introduced, containing:

- a)- 0.1 g of *Bifidobacterium lactis* ID n. LMG P-21384, at a concentration of $1 \cdot 10^{11}$ CFU/g of bacterial strain, equal to $2 \cdot 10^9$ CFU/g of composition;
- b)- 0.1 g of *Lactobacillus casei* subsp. *rhamnosus* ID n. DSM 16605, at a concentration of $1 \cdot 10^{11}$ CFU/g of bacterial strain, equal to $2 \cdot 10^9$ CFU/g of composition;
- c)- 0.1 g of *Lactobacillus plantarum* ID n. LMG P-21020, at a concentration of $1 \cdot 10^{11}$ CFU/g of bacterial strain, equal to $2 \cdot 10^9$ CFU/g of composition;
- d)- FOS-c.c.: 3 g;
- e)- glucose: 1.7 g.

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Composition B

In a single dose sachet, 5 g of granulate for oral use are introduced, containing:

- a)- 0.5 g of *Bifidobacterium lactis* ID n. LMG P-21384, at a concentration of $1 \cdot 10^{12}$ CFU/g of bacterial strain, equal to $1 \cdot 10^{11}$ CFU/g of composition;
- b)- 0.2 g of *Lactobacillus casei* subsp. *rhamnosus* ID n. DSM 16605, at a concentration of $1 \cdot 10^{11}$ CFU/g of bacterial strain, equal to $4 \cdot 10^9$ CFU/g of composition;
- c)- 0.1 g of *Lactobacillus plantarum* ID n. LMG P-21022, at a concentration of $1 \cdot 10^{11}$ CFU/g of bacterial strain, equal to $2 \cdot 10^9$ CFU/g of composition;
- d)- FOS-c.c.: 4 g;
- e)- acesulfame: 0.2 g.

Composition C

In a single dose sachet, 5 g of granulate for oral use are introduced, containing:

- a)- 0.2 g of *Bifidobacterium lactis* ID n. LMG P-21384, at a concentration of $1 \cdot 10^{11}$ CFU/g of bacterial strain, equal to $4 \cdot 10^9$ CFU/g of composition;
- b)- 0.2 g of *Lactobacillus casei* subsp. *rhamnosus* ID n. DSM 16605, at a concentration of $1 \cdot 10^{11}$ CFU/g of bacterial strain, equal to $4 \cdot 10^9$ CFU/g of composition;
- c)- 0.1 g of *Lactobacillus plantarum* ID n. LMG P-21023, at a concentration of $1 \cdot 10^{12}$ CFU/g of bacterial

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strain, equal to $2 \cdot 10^{10}$ CFU/g of composition;

d)- FOS-c.c.: 3.5 g;

e)- 0.5 g of *Lactobacillus delbrueckii* subsp. *bulgaricus* ID n. DSM 16607, at a concentration of $1 \cdot 10^7$ CFU/g of bacterial strain, equal to $1 \cdot 10^6$ CFU/g of composition;

f)- acesulfame: 0,2 g;

g)- vitamin C: 0.3 g.

In another preferred embodiment, the pharmaceutical compositions of the present invention may also contain one or more pharmacologically active substances.

Said pharmacologically active substances can opportunely be formulated in admixture with the other components of the composition, such that they can be taken with a single administration.

Said pharmacologically active substances can also be formulated in a discrete packages, so as to allow an independent administration (if necessary also in different times) of the components, however such that the synergic effect is maintained, depending on the requirements of the patient.

In this case, independent packages are arranged, containing the composition of the present invention and the pharmacologically active substance/s, respectively.

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The discrete packages above-mentioned are then introduced in a proper kit for allowing the patient to sequentially or separately take them, so as to benefit of a opportunely coordinated therapy depending on its own requirements.

By mere way of example, a kit as the one above-mentioned can contain a number of sachets, or capsules, for the oral administration of the compositions of the present invention, in combination with a proper number of doses of antibiotic and/or multivitaminic complex and/or mucolytic, sufficient for a week of therapy.

By way of absolutely non limiting example, in support of the wide application potential of the present invention, the results of a clinical study carried out with one of the preferred compositions of the same are shown hereinbelow.

In a perspective study, conducted in double-blind randomized against placebo, the ability of the composition A of the present invention of improving the organism protection and restoring a normal state of health thereof towards the respiratory infections has been evaluated.

The rational of the clinical trial carried out consists in that the medical literature data show the association existing between the predisposition to the

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influenza like seasonal infections at the expense of the respiratory system (ILI and ARI) and the presence of an insufficient mucosal immunity.

In particular, what observed during the years is an increase of the pathologies above-mentioned in those subjects that, for some reasons (such as for example physical stress, chronic pathologies, ageing) had a low protection from the mucosal immunity.

The study has involved the enrolment of 237 subjects, of which 122 treated with the preparation A and 115 treated with placebo.

The mean age of the two different samples was 35.8 (with standard deviation 15.3 years) and 34.1 (with standard deviation 16.3 years).

To each patient of the first group, the intake per os of the sachet content of granulate of preparation A (by previous dissolution in water or other kind of non-carbonated nor alcoholic beverage) has been prescribed, each morning for 30 consecutive days.

To the placebo group, the intake of the content of a sachet containing 5 g of glucose has been prescribed (with the same administration forms and posology).

The course of the health condition of the enrolled subjects has been followed through the drawing up of a daily diary, in which those attending the study (or

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the sanitary staff put in charge of the control) have reported the daily presence of pathologies at the expense of the respiratory system (for the month of treatment or the two following months).

Incidence, length and subjective gravity of the acute respiratory infections, as well as the course of the intestinal functionality have been examined.

For the comparison between the frequencies of events between the two groups, the chi-square test with the Yates' correction has been used, while those relating to length and gravity of the cases found between the groups the ANOVA (ANalysis Of VAriance) has been used, except for the case of non homogeneity of the variances (evaluated through the Bartlett's test), where it has been substituted by the Kruskal-Wallis's test. For the statistical analysis, the "Epi Info" programme, version 6.04d has been used.

At the same time as the execution of the main trial, the validity of the defense provided to the organism by the symbiotic composition A has been evaluated on another group of subjects, in good health and without any pathologies at the expense of the respiratory system.

On said subjects, the qualitative and quantitative dosage of the secretion at the mucosal level of the

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secretory IgA has been carried out, before, during and for 30 days after the administration of the composition A.

The experimentation has pointed out a statistically significant decrease of the length of the conditions generally considered (-1.32 days; $p=0.016$) and, in particular, for those due to infections of the high respiratory tracts (URTI; -2.83 days; $p=0.034$).

It has also been found a reduction trend for the other considered pathologies categories, such as bronchitis, influenza, common cold, cough.

The analytical determination of the secretory IgA in the salivary samples of the subjects treated with the composition A has pointed out an increasing production of the IgAs starting from the 5th-6th day from the beginning of the treatment, increase which has lasted up to about 4-14 days from the suspension of the same.

This data confirms the effectiveness of the symbiotic composition A for increasing the systemic immune defences and of the mucose of the respiratory system, condition which, among other things, allows to prevent and fight infections caused by influenza and parainfluenza virus.

Also the inspection relating to the intestinal functionality (compromised by the pathologies above-

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mentioned) which, following to the intake of the symbiotic composition has shown an improvement is revealed statistically significant, both in terms of reduction of the swelling and in terms of the regularity of the intestine.

These inspections point out as the regular prolonged intake of a symbiotic pharmaceutical composition according to the present invention is able to affect in a positive way the health of the organism, both in a preventive function and in a therapeutic function, towards the respiratory pathologies and/or infections.

With respect, in particular, to the preventive aspect, it is possible to state that the regular intake of a symbiotic pharmaceutical composition according to the present invention is able to effectively protect the organism from the onset of the pathologies above-mentioned in competition with a traditional influenza vaccine, however without inducing the negative symptoms (for example generalized discomforts, intermittent occasional fever, pain in the bones and articulations and allergic reactions).

Accordingly, the symbiotic compositions of the present invention can also be used for the preparation of a medicament acting as an influenza vaccine.

Furthermore, it has also been pointed out that the in-

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take of a pharmaceutical composition according to the present invention is simultaneously able to improve and/or regularize the intestinal functionality of the organism, often compromised by said pathologies.

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CLAIMS

1. Use of a composition including a mixture of probiotic bacteria, for the preparation of a medicament for the prevention and/or the therapeutic treatment of respiratory pathologies and/or infections.
2. Use according to claim 1, for the simultaneous improvement and/or regularization of the intestinal functionality of the organism.
3. Use according to claim 1, for the preparation of an influenza vaccine.
4. Use according to any one of the claims 1-3, wherein said mixture consists of bacteria belonging to the genus *Lactobacillus* and/or the genus *Bifidobacterium*.
5. Use according to any one of the claims 1-4, wherein said mixture consists in the three following bacterial species: *Bifidobacterium lactis*, *Lactobacillus casei* subsp. *rhamnosus*, *Lactobacillus plantarum*.
6. Use according to claim 5, wherein the bacterial strains belonging to said bacterial species are selected from:
Bifidobacterium lactis, ID n. LMG P-21384;
Lactobacillus casei subsp. *rhamnosus*, ID n. DSM 16605;
Lactobacillus plantarum, ID n. LMG P-21021;

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Lactobacillus plantarum, ID n. LMG P-21020;

Lactobacillus plantarum, ID n. LMG P-21022;

Lactobacillus plantarum, ID n. LMG P-21023.

7. Use according to any one of the preceding claims, wherein said composition further includes at least a prebiotic.

8. Use according to claim 7, wherein said prebiotic is selected from: fructo-oligosaccharides, short-chain fructo-oligosaccharides, inulin, isomalt-oligosaccharides, pectins, galacto-oligosaccharides, arabinogalactan, xylo-oligosaccharides, chitosan-oligosaccharides, glucanmannan, beta-glucans, Konjac, guar, arabic, xanthan gums, modified and resistant starches, polydextrose, D-tagatose; preferably, short-chain fructo-oligosaccharides.

9. Use according to any one of the claims 5-8, wherein said composition further includes at least a probiotic selected among the bacterial species:

Bifidobacterium adolescentis, *Bifidobacterium angulatum*, *Bifidobacterium animalis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium catenulatum*, *Bifidobacterium denticolens*, *Bifidobacterium dentium*, *Bifidobacterium gallicum*, *Bifidobacterium infantis*, *Bifidobacterium inopinatum*, *Bifidobacterium longum*, *Bifidobacterium pseudocatenulatum*, *Enterococcus*

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faecium, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus casei* subsp. *paracasei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus fermentum*, *Lactobacillus GG*, *Lactobacillus pentosus*, *Lactobacillus salivarius*, *Saccharomyces boulardi*, *Streptococcus thermophilus*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *diacetylactis*.

10. Use according to claim 9, wherein said probiotic is selected from the following bacterial strains:

Lactobacillus acidophilus, ID n. LMG P-21381;

Lactobacillus casei subsp. *paracasei*, ID n. LMG P-21380;

Lactobacillus pentosus, ID n. LMG P-21019;

Lactobacillus delbrueckii subsp. *bulgaricus*, ID n. DSM 16607;

Lactobacillus delbrueckii subsp. *bulgaricus*, ID n. DSM 16606.

11. Use according to any one of the preceding claims, wherein said composition further includes at least a pharmacologically active substance.

12. Use according to claim 11, wherein said pharmacologically active substance is directly present in admixture with the other components of said composition or is formulated and packaged separately from the same.

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13.. Composition for one of the uses described in any one of the claims 1-3, including a mixture of probiotic bacteria consisting in the following three bacterial species: *Bifidobacterium lactis*, *Lactobacillus casei* subsp. *rhamnosus*, *Lactobacillus plantarum*.

14. Composition according to claim 13, wherein the bacterial strains belonging to said bacterial species are selected from:

Bifidobacterium lactis, ID n. LMG P-21384;

Lactobacillus casei subsp. *rhamnosus*, ID n. DSM 16605;

Lactobacillus plantarum, ID n. LMG P-21021;

Lactobacillus plantarum, ID n. LMG P-21020;

Lactobacillus plantarum, ID n. LMG P-21022;

Lactobacillus plantarum, ID n. LMG P-21023.

15. Composition according to any one of the claims 13-14, wherein said composition further includes at least a prebiotic.

16. Composition according to claim 15, wherein said prebiotic is selected from: fructo-oligosaccharides, short-chain fructo-oligosaccharides, inulin, isomalt-oligosaccharides, pectins, galacto-oligosaccharides, arabinogalactan, xylo-oligosaccharides, chitosan-oligosaccharides, glucanmannan, beta-glucans, Konjac, guar, arabic, xanthan gums, modified and resistant starches, polydextrose, D-tagatose; preferably, short-

-28-

chain fructo-oligosaccharides.

17. Composition according to any one of the claims 13-16, wherein said composition further includes at least a probiotic selected among the bacterial species:

Bifidobacterium adolescentis, *Bifidobacterium angulatum*, *Bifidobacterium animalis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium catenulatum*, *Bifidobacterium denticolens*, *Bifidobacterium dentium*, *Bifidobacterium gallicum*, *Bifidobacterium infantis*, *Bifidobacterium inopinatum*, *Bifidobacterium longum*, *Bifidobacterium pseudocatenulatum*, *Enterococcus faecium*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus casei subsp. paracasei*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Lactobacillus fermentum*, *Lactobacillus GG*, *Lactobacillus pentosus*, *Lactobacillus salivarius*, *Saccharomyces boulardi*, *Streptococcus thermophilus*, *Lactococcus lactis subsp. lactis*, *Lactococcus lactis subsp. diacetylactis*.

18. Composition according to claim 17, wherein said probiotic is selected from the bacterial strains:

Lactobacillus acidophilus, ID n. LMG P-21381;

Lactobacillus casei subsp. paracasei, ID n. LMG P-21380;

Lactobacillus pentosus, ID n. LMG P-21019;

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Lactobacillus delbrueckii subsp. *bulgaricus*, ID n. DSM 16607;

Lactobacillus delbrueckii subsp. *bulgaricus*, ID n. DSM 16606.

19. Composition according to any one of the claims 13-18, wherein said composition further includes at least a pharmacologically active substance.

20. Composition according to claim 19, wherein said pharmacologically active substance is directly present in admixture with the other components of said composition, or is formulated and packaged separately from the same.

21. Composition according to claim 13, wherein said three bacterial species of said mixture are present in a mutual weight ratio between 0.1:1 to 10:1; preferably in a mutual weight ratio between 0.5:1 to 2:1; more preferably in a ratio of about 1:1.

22. Composition according to claim 13, wherein each single bacterial species is dosed in a quantity between $1 \cdot 10^6$ UFC/dose to $1 \cdot 10^{12}$ UFC/dose; preferably, from $1 \cdot 10^9$ UFC/dose to $1 \cdot 10^{11}$ UFC/dose.

23. Composition according to any one of the claims 15-16, wherein said prebiotic is present in a weight ratio between 0:1 to 100:1, based on the total quantity of said three bacterial species; preferably, in a

-30-

weight ratio between 2:1 to 15:1; more preferably, from 5:1 to 10:1.

24. Composition according to any one of the claims 13-23, including a granulate for oral use, containing:

a)- 0.1 g of *Bifidobacterium lactis* ID n. LMG P-21384, at a concentration of $1 \cdot 10^{11}$ CFU/g of bacterial strain, equal to $2 \cdot 10^9$ CFU/g of composition;

b)- 0.1 g of *Lactobacillus casei* subsp. *rhamnosus* ID n. DSM 16605, at a concentration of $1 \cdot 10^{11}$ CFU/g of bacterial strain, equal to $2 \cdot 10^9$ CFU/g of composition;

c)- 0.1 g of *Lactobacillus plantarum* ID n. LMG P-21020, at a concentration of $1 \cdot 10^{11}$ CFU/g of bacterial strain, equal to $2 \cdot 10^9$ CFU/g of composition;

d)- FOS-c.c.: 3 g;

e)- glucose: 1.7 g.

25. Composition according to any one of the claims 13-24, further including at least a pharmacologically active substance, wherein said pharmacologically active substance is directly present in admixture with the other components of said composition or is formulated and packaged separately from the same.

26. Kit including, as components:

- at least a composition according to any one of the claims 13-24;
- at least a pharmacologically active substance;

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separately packaged, for the independent, consecutive
or not, administration of said components.

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

DSMZ

Deutsche Sammlung von
Mikroorganismen und
Zellkulturen GmbH



INTERNATIONAL FORM

ANIDRAL S.R.L.
Via Pietro Custodi 12
28100 Novara
Italy

PCT/IB 05 / 03214

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: LR 04	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 16605
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I. above was accompanied by: (X) a scientific description (X) a proposed taxonomic designation (Mark with a cross where applicable).	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2004-07-20 (Date of the original deposit)¹.	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I. above was received by this International Depositary Authority on _____ (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on _____ (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): <i>V. Wehls</i> Date: 2004-07-26

¹ Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

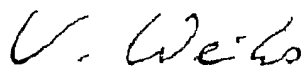
BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE



INTERNATIONAL FORM

ANIDRAL S.R.L.
Via Pietro Custodi 12
28100 Novara
Italy

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: ANIDRAL S.R.L. Via Pietro Custodi 12 Address: 28100 Novara Italy	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 16605 Date of the deposit or the transfer ¹ : 2004-07-20
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 2004-07-21 On that date, the said microorganism was ☒ ² viable ☐ ² no longer viable	
IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED ⁴	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):  Date: 2004-07-26

- ¹ Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
² In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
³ Mark with a cross the applicable box.
⁴ Fill in if the information has been requested and if the results of the test were negative.

(Fill out the details on the screen, print it out, sign it, and send to the DSMZ)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE
STATEMENT IN THE CASE OF AN ORIGINAL DEPOSIT

DSMZ
Deutsche Sammlung von
Mikroorganismen und
Zellkulturen GmbH

pursuant to Rule 6.1

To:
DSMZ-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1b
D-38124 Braunschweig
GERMANY

To be filled in by the Depositary Authority

DSMZ-Accession Number :

Date culture received:

BACTERIA/FUNGI ¹

THE UNDERSIGNED HEREBY DEPOSITS UNDER THE BUDAPEST TREATY THE MICROORGANISM IDENTIFIED HEREUNDER AND UNDERTAKES NOT TO WITHDRAW THE DEPOSIT FOR THE PERIOD SPECIFIED IN RULE 9.1 ²

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference ³ : <u>LR 04</u>	The culture to be deposited is : ☒ a pure culture ☐ a mixture of microorganisms
Taxonomic designation ⁴ : <u>Lactobacillus casei subsp. rhamnosus</u>	☐
II. CONDITIONS FOR CULTIVATION	
Medium: <u>MRS broth</u> <u>Difco ref 288130</u>	pH before sterilisation: <u>7,00 ± 0,2</u> Sterilisation <u>15</u> min at <u>121</u> °C pH after sterilisation: <u>6,5 ± 0,2</u> Oxygen relationship: ☐ aerobic ☒ microaerophilic ☐ obligate anaerobic Specific gaseous requirements:
Incubation temperature: <u>37</u> °C Incubation time: <u>16 ± 1 hours</u> Short term storage at: <u>5</u> °C Interval of transfer: <u>5-7 days</u>	

¹ The DSMZ only accepts for deposit microorganisms which belong to risk group 1 or 2, according to the council directive 2000/54/EEC on the protection of workers from risks related to exposure to biological agents at work (OJ No L 262/21 of 13.10.2000) and can be classified as S1 or S2 organisms according to -Gesetz zur Regelung der Gentechnik" (BGBl. I, pp. 2067-2083 of 21.12.1993), last changed on 16.08.2002 (BGBl. I, pp. 3220-3244) and according to EEC Directive 2000/54/EC on the Protection of Workers from Risks Related to Exposure to Biological Agents at Work (OJ No. L262, pp. 21-45 of 18.09.2000).

² This form may also be used if the undersigned converts into a deposit under the Budapest Treaty the deposit of a microorganism that he or his predecessor in title has already deposited, outside the Budapest Treaty, with the same depositary institution either before (Rule 6.4(d)) or after the acquisition by that institution of the status of international depositary authority.

³ Number, symbols etc., given to the microorganism by the depositor.

⁴ It is strongly recommended to indicate the taxonomic designation and/or scientific description (see under VII.) of the microorganism.

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

DSMZ

Deutsche Sammlung von
Mikroorganismen und
Zellkulturen GmbH

PCT/IB 05 / 03214

INTERNATIONAL FORM

ANIDRAL S.R.L.

Via Pietro Custodi 12

28100 Novara

Italy

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: LDB 01	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 16506
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I. above was accompanied by: (X) a scientific description (X) a proposed taxonomic designation (Mark with a cross where applicable).	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2004-07-20 (Date of the original deposit).	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): <i>V. Wetts</i> Date: 2004-07-26

¹ Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

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

DSMZ
Deutsche Sammlung von
Mikroorganismen und
Zellkulturen GmbH

INTERNATIONAL FORM PCT/IB 05 / 03214

ANIDRAL S.R.L.
Via Pietro Custodi 12
28100 Novara
Italy

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: ANIDRAL S.R.L. Via Pietro Custodi 12 Address: 28100 Novara Italy	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 16606 Date of the deposit or the transfer: 2004-07-20
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 2004-07-21 On that date, the said microorganism was ☒ ¹ viable ☐ ² no longer viable	
IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED ⁴	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Bratunischweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):  Date: 2004-07-26

- ¹ Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
- ² In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
- ³ Mark with a cross the applicable box.
- ⁴ Fill in if the information has been requested and if the results of the test were negative.

2. Fill out the details on the screen, print it out, sign it, and send to the DSMZ

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE
STATEMENT IN THE CASE OF AN ORIGINAL DEPOSIT

DSMZ
Deutsche Sammlung von
Mikroorganismen und
Zellkulturen GmbH

PCT/IB 05 / 03214

pursuant to Rule 6.1

To
DSMZ-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1b
D-38124 Braunschweig
GERMANY

To be filled in by the Depositary Authority

DSMZ-Accession Number:

Date culture received:

BACTERIA/FUNGI¹

THE UNDERSIGNED HEREBY DEPOSITS UNDER THE BUDAPEST TREATY THE MICROORGANISM IDENTIFIED HEREUNDER
AND UNDERTAKES NOT TO WITHDRAW THE DEPOSIT FOR THE PERIOD SPECIFIED IN RULE 9.1²

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference: <u>LDB 01</u>	The culture to be deposited is: ☒ a pure culture ☐ a mixture of microorganisms
Taxonomic designation: <u>Lactobacillus delbrueckii subsp. bulgaricus</u>	
II. CONDITIONS FOR CULTIVATION	
Medium: <u>MRS broth</u> <u>Difco ref 288130</u>	pH before sterilisation: <u>7.00 ± 0.2</u> Sterilisation <u>15 min</u> at <u>121 °C</u> pH after sterilisation: <u>6.5 ± 0.2</u> Oxygen relationship: ☐ aerobic ☒ microaerophilic ☐ obligate anaerobic Specific gaseous requirements:
	Incubation temperature: <u>37 °C</u> Incubation time: <u>16 ± 1 hours</u> Short term storage at: <u>5 °C</u> Interval of transfer: <u>5-7 days</u>

¹ The DSMZ only accepts for deposit microorganisms which belong to risk group 1 or 2, according to the council directive 2000/54/EEC on the protection of workers from risks related to exposure to biological agents at work (OJ No L 262/21 of 13.10.2000) and can be classified as S1 or S2 organisms according to "Gesetz zur Regelung der Gentechnik" (BGBI. I, pp. 2067-2083 of 21.12.1993), last changed on 16.08.2002 (BGBI. I, pp. 3220-3244) and according to EEC Directive 2000/54/EC on the Protection of Workers from Risks Related to Exposure to Biological Agents at Work (OJ No. L262, pp. 21-45 of 18.09.2000).

² This form may also be used if the undersigned converts into a deposit under the Budapest Treaty the deposit of a microorganism that he or his predecessor in title has already deposited, outside the Budapest Treaty, with the same depositary institution either before (Rule 6.4(d)) or after the acquisition by that institution of the status of international depositary authority.

³ Number, symbols etc., given to the microorganism by the depositor.

⁴ It is strongly recommended to indicate the taxonomic designation and/or scientific description (see under VII.) of the microorganism.

⁵ Mark with a cross if additional information is given on an attached sheet

FOR PATENT PURPOSES ONLY!

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



INTERNATIONAL FORM

ANIDRAL S.R.L.
Via Pietro Custodi 12
28100 Novara
Italy

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: LDB 02	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 16607
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I. above was accompanied by: (X) a scientific description (X) a proposed taxonomic designation (Mark with a cross where applicable).	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2004-07-20 (Date of the original deposit)¹.	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on _____ (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on _____ (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): <i>V. Welts</i> Date: 2004-07-26

¹ Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

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



INTERNATIONAL FORM

ANIDRAL S.R.L.
Via Pietro Custodi 12
28100 Novara
Italy

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: ANIDRAL S.R.L. Via Pietro Custodi 12 Address: 28100 Novara Italy	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 16607 Date of the deposit or the transfer ¹ : 2004-07-20
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 2004-07-21 On that date, the said microorganism was ☒ ² viable ☐ ² no longer viable	
IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED ⁴	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):  Date: 2004-07-26

¹ Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

² In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.

³ Mark with a cross the applicable box.

⁴ Fill in if the information has been requested and if the results of the test were negative.

(fill out the details on the screen, print it out, sign it, and send to the DSMZ)

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE
STATEMENT IN THE CASE OF AN ORIGINAL DEPOSIT

DSMZ
Deutsche Sammlung von
Mikroorganismen und
Zellkulturen GmbH

pursuant to Rule 6.1

To
DSMZ-DEUTSCHE SAMMLUNG VON
MIKROORGANISMEN UND ZELLKULTUREN GmbH
Mascheroder Weg 1b
D-38124 Braunschweig
GERMANY

To be filled in by the Depositary Authority

DSMZ-Accession Number :

Date culture received:

BACTERIA/FUNGI ¹⁾

THE UNDERSIGNED HEREBY DEPOSITS UNDER THE BUDAPEST TREATY THE MICROORGANISM IDENTIFIED HEREUNDER
AND UNDERTAKES NOT TO WITHDRAW THE DEPOSIT FOR THE PERIOD SPECIFIED IN RULE 9.1 ²⁾

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference: <u>LDB02</u>	The culture to be deposited is: ☒ a pure culture ☐ a mixture of microorganisms
Taxonomic designation: <u>Lactobacillus delbrueckii sub. bulgaricus</u>	
II. CONDITIONS FOR CULTIVATION	
Medium: <u>MRS broth</u> <u>DIFCO ref. 288130</u>	pH before sterilisation: <u>7.00 ± 0.2</u> Sterilisation <u>15 min</u> at <u>121 °C</u> pH after sterilisation: <u>6.5 ± 0.2</u> Oxygen relationship: ☐ aerobic ☒ microaerophilic ☐ obligate anaerobic Specific gaseous requirements:
	Incubation temperature: <u>37 °C</u> Incubation time: <u>16 hours ± 1</u> Short term storage at: <u>5 °C</u> Interval of transfer: <u>5-7 days</u>

¹⁾ The DSMZ only accepts for deposit microorganisms which belong to risk group 1 or 2, according to the council directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work (OJ No L 262/21 of 13.10.2000) and can be classified as S1 or S2 organisms according to "Gesetz zur Regelung der Gentechnik" (BGBl. I, pp. 2067-2083 of 21.12.1993), last changed on 16.08.2002 (BGBl. I, pp. 3220-3244) and according to EEC Directive 2000/54/EC on the Protection of Workers from Risks Related to Exposure to Biological Agents at Work (OJ No. L262, pp. 21-45 of 18.09.2000).

²⁾ This form may also be used if the undersigned converts into a deposit under the Budapest Treaty the deposit of a microorganism that he or his predecessor in title has already deposited, outside the Budapest Treaty, with the same depositary institution either before (Rule 6.4(d)) or after the acquisition by that institution of the status of international depositary authority.

³⁾ Number, symbols etc., given to the microorganism by the depositor.

⁴⁾ It is strongly recommended to indicate the taxonomic designation and/or scientific description (see under VII.) of the microorganism.

LMG-COLLECTION

Page 1 of Form BCCM/LMG/BP/4/01-105 Receipt in the case of an original deposit

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure

Receipt in the case of an original deposit issued pursuant to Rule 7.1 by the International Depositary Authority BCCM/LMG identified at the bottom of next page

International Form BCCM/LMG/BP/4/01-105

To: Name of the depositor : Laboratorio Microbiologico Grana Provolone SRL

Address : Via P. Custodi N. 12
I-28100 Novara
Italy

I. Identification of the microorganism:

I.1 Identification reference given by the depositor:

Lactobacillus pentosus 9 /1 ei

I.2 Accession number given by the International Depositary Authority:

LMG P-21019

II. Scientific description and/or proposed taxonomic designation

The microorganism identified under I above was accompanied by :

(mark with a cross the applicable box(es)):

- ☒ a scientific description
☒ a proposed taxonomic designation

III. Receipt and acceptance

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on (date of original deposit)

October 16, 2001

IV. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):



Dr. D. Janssens, curator IDA

Date :

October 30, 2001

LMG-COLLECT IN

Page 1 of Form BCCM/LMG/BP/9/01-105... Viability statement

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure**Viability statement issued pursuant to Rule 10.2 by the International Depositary Authority BCCM/LMG identified on the following page*****International Form BCCM/LMG/BP/9/01-105.....*****To: Party to whom the viability statement is issued:**

Name : Laboratorio Microbiologico Grana Provolone SRL
Attn: Luigi Dell'era

Address : Via P. Custodi N. 12
I-28100 Novara
Italy

I. Depositor:

I.1 Name : Laboratorio Microbiologico Grana Provolone SRL

I.2 Address : Via P. Custodi N. 12
I-28100 Novara
Italy

II. Identification of the microorganism**II.1 Accession number given by the International Depositary Authority:**

LMG P-21019

II.2 Date of the original deposit (or where a new deposit or a transfer has been made, the most recent relevant date)

October 16, 2001

III. Viability statement.**The viability of the microorganism identified under II above was tested on**

October 17, 2001

(Give date. In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test).

On that date, the said microorganism was: (mark the applicable box with a cross)☒ viable☐ no longer viable

II. Scientific description and/or proposed taxonomic designation

The microorganism identified under I above was accompanied by :

(mark with a cross the applicable box(es)):

- ☒ a scientific description
☒ a proposed taxonomic designation

III. Receipt and acceptance

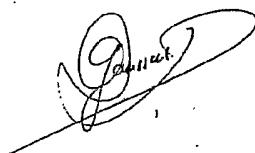
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on (date of original deposit) :

October 16, 2001

IV. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):



Dr. D. Janssens, curator IDA

Date :

October 30, 2001

LMG-COLLECTIO

Page 2 of Form BCCM/LMG/BP/9/01-105 Viability statement

IV. Conditions under which the viability test has been performed:

(Fill in if the information has been requested and if the results of the test were negative).

V. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or
of authorized official(s) :



Dr. D. Janssens, curator IDA

Date : October 30, 2001

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION

Page 1 of Form BCCM/LMG/BP/4/.....01-106 Receipt in the case of an original deposit

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure

Receipt in the case of an original deposit issued pursuant to Rule 7.1 by the
International Depositary Authority BCCM/LMG identified at the bottom of next page

International Form BCCM/LMG/BP/4/...01-106...

To: Name of the depositor : Laboratorio Microbiologico Grana Provolone SRL

Address : Via P. Custodi N. 12
I-28100 Novara
Italy

I. Identification of the microorganism:

I.1 Identification reference given by the depositor:

Lactobacillus plantarum 776 /1 bi

I.2 Accession number given by the International Depositary Authority:

LMG P-21020

II. Scientific description and/or proposed taxonomic designation

The microorganism identified under I above was accompanied by :

(mark with a cross the applicable box(es)):

- ☒ a scientific description
☒ a proposed taxonomic designation

III. Receipt and acceptance

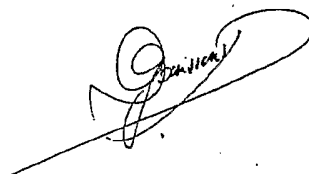
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on (date of original deposit)

October 16, 2001

IV. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):



Dr. D. Janssens, curator IDA

Date :

October 30, 2001

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS
LMG-COLLECTION
Page 1 of Form BCCM/LMG/BP/9/.....01-106 Viability statement

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure

Viability statement issued pursuant to Rule 10.2 by the International Depositary
Authority BCCM/LMG identified on the following page

International Form BCCM/LMG/BP/9/.....01-106

To: Party to whom the viability statement is issued:

Name : Laboratorio Microbiologico Grana Provolone SRL
Attn: Luigi Dell'era
Address : Via P. Custodi N. 12
I-28100 Novara
Italy

I. Depositor:

I.1 Name : Laboratorio Microbiologico Grana Provolone SRL
I.2 Address : Via P. Custodi N. 12
I-28100 Novara
Italy

II. Identification of the microorganism

II.1 Accession number given by the International Depositary Authority:

LMG P-21020

II.2 Date of the original deposit (or where a new deposit or a transfer has been made, the most recent relevant date) :

October 16, 2001

III. Viability statement.

The viability of the microorganism identified under II above was tested on

: October 17, 2001

(Give date. In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test).

On that date, the said microorganism was: (mark the applicable box with a cross)

☒ viable

☐ no longer viable

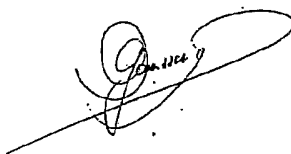
IV. Conditions under which the viability test has been performed:

(Fill in if the information has been requested and if the results of the test were negative).

V. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) :



Dr. D. Janssens, curator IDA

Date : October 30, 2001

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION
Page 1 of Form BCCM/LMG/BP/4/.....01-107

PCT/IB 05 / 03214

**Budapest Treaty on the International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure**

Receipt in the case of an original deposit issued pursuant to Rule 7.1 by the
International Depositary Authority BCCM/LMG identified at the bottom of next page

01-107
International Form BCCM/LMG/BP/4/.....

To: Name of the depositor : Laboratorio Microbiologico Grana Provolone SRL

Address : Via P. Custodi N. 12
I-28100 Novara
Italy

I. Identification of the microorganism:

I.1 Identification reference given by the depositor:

Lactobacillus plantarum 476LL 20 bi

I.2 Accession number given by the International Depositary Authority:

LMG P-21021

II. Scientific description and/or proposed taxonomic designation

The microorganism identified under I above was accompanied by :

(mark with a cross the applicable box(es)):

☒ a scientific description

☒ a proposed taxonomic designation

III. Receipt and acceptance

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on (date of original deposit)

October 16, 2001

IV. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):



Dr. D. Janssens, curator IDA

Date : October 30, 2001

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION 01-107
Page 1 of Form BCCM/LMG/BP/9/..... Viability statement

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure

Viability statement Issued pursuant to Rule 10.2 by the International Depositary Authority BCCM/LMG Identified on the following page

International Form BCCM/LMG/BP/9/.....01-107.....

To: Party to whom the viability statement is issued:

Name : Laboratorio Microbiologico Grana Provolone SRL
Attn: Luigi Dell'era

Address : Via P. Custodi N. 12
I-28100 Novara
Italy

I. Depositor:

I.1 Name : Laboratorio Microbiologico Grana Provolone SRL

I.2 Address : Via P. Custodi N. 12
I-28100 Novara
Italy

II. Identification of the microorganism

II.1 Accession number given by the International Depositary Authority:

LMG P-21021

II.2 Date of the original deposit (or where a new deposit or a transfer has been made, the most recent relevant date) :

October 16, 2001

III. Viability statement.

The viability of the microorganism identified under II above was tested on

: October 17, 2001

(Give date. In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test).

On that date, the said microorganism was: (mark the applicable box with a cross)

- ☒ viable
☐ no longer viable

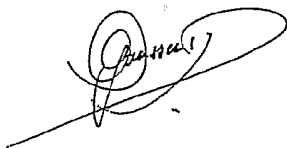
IV. Conditions under which the viability test has been performed:

(Fill in if the information has been requested and if the results of the test were negative).

V. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) :



Dr. D. Janssens, curator IDA

Date : October 30, 2001

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION
Page 1 of Form BCCM/LMG/BP/4/01-108... Receipt in the case of an original deposit

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure

Receipt in the case of an original deposit issued pursuant to Rule 7.1 by the
International Depositary Authority BCCM/LMG identified at the bottom of next page

International Form BCCM/LMG/BP/4/01-108...

To: Name of the depositor : Laboratorio Microbiologico Grana Provolone SRL

Address : Via P. Custodi N. 12
I-28100 Novara
Italy

I. Identification of the microorganism:

I.1 Identification reference given by the depositor:

Lactobacillus platarum PR ci

I.2 Accession number given by the International Depositary Authority:

LMG P-21022

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION

Page 2 of Form BCCM/LMG/BP/4/...01-108. Receipt in the case of an original deposit

II. Scientific description and/or proposed taxonomic designation

The microorganism identified under I above was accompanied by :

(mark with a cross the applicable box(es)):

☒ a scientific description

☒ a proposed taxonomic designation

III. Receipt and acceptance

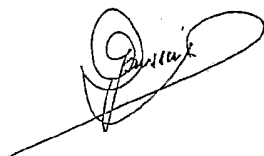
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on (date of original deposit) :

October 16, 2001

IV. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):



Dr. D. Janssens, curator IDA

Date :

October 30, 2001

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION

Page 1 of Form BCCM/LMG/BP/9/.....01-108 Viability statement

**Budapest Treaty on the International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure**

**Viability statement Issued pursuant to Rule 10.2 by the International Depositary
Authority BCCM/LMG Identified on the following page**

International Form BCCM/LMG/BP/9/.....01-108

To: Party to whom the viability statement is issued:

Name : Laboratorio Microbiologico Grana Provolone SRL
Attn: Luigi Dell'era

Address : via P. Custodi N. 12
I-28100 Novara
Italy

I. Depositor:

I.1 Name : Laboratorio Microbiologico Grana Provolone SRL

I.2 Address : Via P. Custodi N. 12
I-28100 Novara
Italy

II. Identification of the microorganism

II.1 Accession number given by the International Depositary Authority:

LMG P-21022

II.2 Date of the original deposit (or where a new deposit or a transfer has been made, the most recent relevant date) :

October 16, 2001

III. Viability statement.

The viability of the microorganism identified under II above was tested on

: October 17, 2001

(Give date. In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test).

On that date, the said microorganism was: (mark the applicable box with a cross)

☒ viable

☐ no longer viable

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION

Page 2 of Form BCCM/LMG/BP/9/01-108 Viability statement

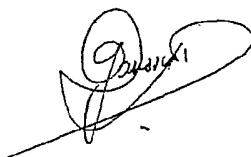
IV. Conditions under which the viability test has been performed:

(Fill in if the information has been requested and if the results of the test were negative).

V. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or
of authorized official(s) :



Dr. D. Janssens, curator IDA

Date : October 30, 2001

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION 01-109
Page 1 of Form BCCM/LMG/BP/4/..... Receipt in the case of an original deposit

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure

Receipt in the case of an original deposit issued pursuant to Rule 7.1 by the
International Depositary Authority BCCM/LMG Identified at the bottom of next page

International Form BCCM/LMG/BP/4/.....01-109...

To: Name of the depositor : Laboratorio Microbiologico Grana Provolone SRL

Address : Via P. Custodi N. 12
I-28100 Novara
Italy

Identification of the microorganism:

I.1 Identification reference given by the depositor:

Lactobacillus plantarum 776 /2 hi

I.2 Accession number given by the International Depositary Authority:

LMG P-21023

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION

Page 2 of Form BCCM/LMG/BP/4/...01-109. Receipt in the case of an original deposit

II. Scientific description and/or proposed taxonomic designation

The microorganism identified under I above was accompanied by :

(mark with a cross the applicable box(es)):

☒ a scientific description☒ a proposed taxonomic designation

III. Receipt and acceptance

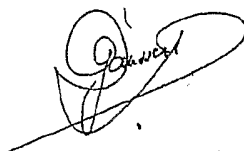
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on (date of original deposit)

October 16, 2001

IV. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):



Dr. D. Janssens, curator IDA

Date :

October 30, 2001

LMG-COLLECT_N

Page 1 of Form BCCM/LMG/BP/9/...01-109... Viability statement

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure

Viability statement issued pursuant to Rule 10.2 by the International Depositary Authority BCCM/LMG identified on the following page

International Form BCCM/LMG/BP/9/...01-109...

To: Party to whom the viability statement is issued:

Name : Laboratorio Microbiologico Grana Provolone SRL
Attn: Luigi Dell'era

Address : Via P. Custodi N. 12
I-28100 Novara
Italy

I. Depositor:

I.1 Name : Laboratorio Microbiologico Grana Provolone SRL

I.2 Address : Via P. Custodi N. 12
I-28100 Novara
Italy

II. Identification of the microorganism

II.1 Accession number given by the International Depositary Authority:

LMG P- 21023

II.2 Date of the original deposit (or where a new deposit or a transfer has been made, the most recent relevant date) :

October 16, 2001

III. Viability statement.

The viability of the microorganism identified under II above was tested on

: October 17, 2001

(Give date. In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test).

On that date, the said microorganism was: (mark the applicable box with a cross)

☒ viable

☐ no longer viable

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION
Page 2 of Form BCCM/LMG/BP/9/01-109... Viability statement

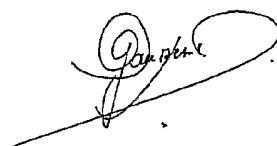
IV. Conditions under which the viability test has been performed:

(Fill in if the information has been requested and if the results of the test were negative).

V. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or
of authorized official(s) :



Dr. D. Janssens, curator IDA

Date : October 30, 2001

BELGIAN COÖRDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION

Page 1 of Form BCCM/LMG/BP/4/.....02-111 Receipt in the case of an original deposit

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure

Receipt in the case of an original deposit issued pursuant to Rule 7.1 by the
International Depositary Authority BCCM/LMG identified at the bottom of next page

International Form BCCM/LMG/BP/4/.....02-111

To: Name of the depositor : Anidral Srl

Address : Via P. Custodi N. 12
28100 Novara - Italy

I. Identification of the microorganism:

I.1 Identification reference given by the depositor:

181A/3 aiai

I.2 Accession number given by the International Depositary Authority:

LMG P-21380

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION
Page 2 of Form BCCM/LMG/BP/4/.....02-111 Receipt in the case of an original deposit

II. Scientific description and/or proposed taxonomic designation

The microorganism identified under I above was accompanied by :

(mark with a cross the applicable box(es)):

- ☒ a scientific description
☒ a proposed taxonomic designation

III. Receipt and acceptance

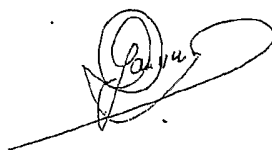
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on (date of original deposit)

January 31, 2002

IV. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):



Dr. D. Janssens, Curator I.D.A.

Date : February 14th, 2002

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION

Page 1 of Form BCCM/LMG/BP/9/...02-111... Viability statement

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure

Viability statement Issued pursuant to Rule 10.2 by the International Depositary
Authority BCCM/LMG Identified on the following page

International Form BCCM/LMG/BP/9/...02-111...

To: Party to whom the viability statement is issued:

Name : Dr. Cesare Schiavi - Amministratore unico

Address : Anidral Srl
Via P. Custodi N. 12
28100 Novara - Italy

I. Depositor:

I.1 Name : Anidral Srl

I.2 Address : Via P. Custodi N. 12
28100 Novara - Italy

II. Identification of the microorganism

II.1 Accession number given by the International Depositary Authority:

LMG P-21380

II.2 Date of the original deposit (or where a new deposit or a transfer has been made, the most recent relevant date) :

January 31, 2002

III. Viability statement.

The viability of the microorganism identified under II above was tested on

: January 31, 2002

(Give date. In the cases referred to in Rule 10.2(a)(ii) and (iii); refer to the most recent viability test).

On that date, the said microorganism was: (mark the applicable box with a cross)

☒ viable

☐ no longer viable

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION

Page 2 of Form BCCM/LMG/BP/9/.....02-111 Viability statement

IV. Conditions under which the viability test has been performed:

(Fill in if the information has been requested and if the results of the test were negative).

V. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or
of authorized official(s) :



Dr. D. Janssens, Curator I.D.A.

Date

February 14th, 2002

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure

Receipt in the case of an original deposit issued pursuant to Rule 7.1 by the
International Depositary Authority BCCM/LMG identified at the bottom of next page

*International Form BCCM/LMG/BP/4/.....*⁰²⁻¹¹²

To: Name of the depositor : Anidral Srl

Address : Via P. Custodi N. 12
28100 Novara - Italy

I. Identification of the microorganism:

I.1 Identification reference given by the depositor:

192A/1 aiaf

I.2 Accession number given by the International Depositary Authority:

LMG P-21381

II. Scientific description and/or proposed taxonomic designation **PCT/IB 05 / 0321**

The microorganism identified under I above was accompanied by :

(mark with a cross the applicable box(es)):

- ☒ a scientific description
☒ a proposed taxonomic designation

III. Receipt and acceptance

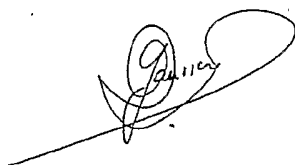
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on (date of original deposit) :

January 31, 2002

IV. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):



Dr. D. Janssens, Curator I.D.A.

Date : February 14th, 2002

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION

Page 1 of Form BCCM/LMG/BP/9/.....02-112 Viability statement

PCT/IB 05 / 03214

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure

Viability statement Issued pursuant to Rule 10.2 by the International Depositary Authority BCCM/LMG Identified on the following page

International Form BCCM/LMG/BP/9/02-112....

To: Party to whom the viability statement is issued:

Name : Dr. Cesare Schiavi - Amministratore unico

Address : Anidral Srl
Via P. Custodi N. 12
28100 Novara - Italy

I. Depositor:

I.1 Name : Anidral Srl

I.2 Address : Via P. Custodi N. 12
28100 Novara - Italy

II. Identification of the microorganism

II.1 Accession number given by the International Depositary Authority:

LMG P-21381

II.2 Date of the original deposit (or where a new deposit or a transfer has been made, the most recent relevant date) :

January 31, 2002

III. Viability statement.

The viability of the microorganism Identified under II above was tested on

: January 31, 2002

(Give date. In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test).

On that date, the said microorganism was: (mark the applicable box with a cross)

☒ viable

☐ no longer viable

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION
Page 2 of Form BCCM/LMG/BP/9/02-112 Viability statement

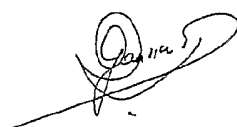
IV. Conditions under which the viability test has been performed:

(Fill in if the information has been requested and if the results of the test were negative).

V. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or
of authorized official(s) :



Dr. D. Janssens, Curator I.D.A.

Date : February 14th, 2002

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION

Page 1 of Form BCCM/LMG/BP/4/.....02-115 Receipt in the case of an original deposit

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure

Receipt in the case of an original deposit issued pursuant to Rule 7.1 by the
International Depositary Authority BCCM/LMG identified at the bottom of next page

International Form BCCM/LMG/BP/4/.....02-115

To: Name of the depositor : Anidral Srl

Address : Via P. Custodi N. 12
28100 Novara - Italy

I. Identification of the microorganism:

I.1 Identification reference given by the depositor:

32A/3 aiai

I.2 Accession number given by the International Depositary Authority:

LMG P-21384

II. Scientific description and/or proposed taxonomic designation

The microorganism identified under I above was accompanied by :

(mark with a cross the applicable box(es)):

- ☒ a scientific description
☒ a proposed taxonomic designation

III. Receipt and acceptance

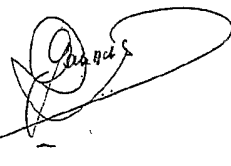
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on (date of original deposit) :

January 31, 2002

IV. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):



Dr. D. Janssens, Curator I.D.A.

Date : February 14th, 2002

BELGIAN COORDINATED COLLECTIONS OF MICROORGANISMS - BCCM
LMG-COLLECTION

Page 1 of Form BCCM/LMG/BP/9/.....02-115 Viability statement

Budapest Treaty on the International Recognition of the Deposit of Microorganisms for
the Purposes of Patent Procedure

Viability statement Issued pursuant to Rule 10.2 by the International Depositary
Authority BCCM/LMG Identified on the following page

International Form BCCM/LMG/BP/9/.....02-115

To: Party to whom the viability statement is issued:

Name : Dr. Cesare Schiavi - Amministratore unico

Address : Anidral Srl
Via P. Custodi N. 12
28100 Novara - Italy

I. Depositor:

I.1 Name : Anidral Srl

I.2 Address : Via P. Custodi N. 12
28100 Novara - Italy

II. Identification of the microorganism

II.1 Accession number given by the International Depositary Authority:

LMG P-21384

II.2 Date of the original deposit (or where a new deposit or a transfer has been made, the most recent relevant date) :

January 31, 2002

III. Viability statement.

The viability of the microorganism identified under II above was tested on

: January 31, 2002

(Give date. In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test).

On that date, the said microorganism was: (mark the applicable box with a cross)

☒ viable

☐ no longer viable

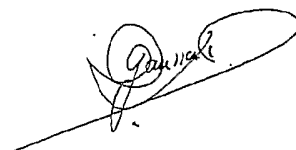
IV. Conditions under which the viability test has been performed:

(Fill in if the information has been requested and if the results of the test were negative).

V. International Depositary Authority

Belgian Coordinated Collections of Microorganisms (BCCM)
Laboratorium voor Microbiologie - Bacteriënverzameling (LMG)
Universiteit Gent
K.L. Ledeganckstraat 35
B-9000 Gent, Belgium

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s) :



Dr. D. Janssens, Curator I.D.A.

Date : February 14th, 2002

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2005/003214

A. CLASSIFICATION OF SUBJECT MATTER

A61K35/74 A61P11/00 A61P31/16

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

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

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

EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 202 02 562 U1 (ORTHOMOL PHARMAZEUTISCHE VERTRIEBS GMBH) 23 May 2002 (2002-05-23)	1-12
Y	page 9 - page 10; claims 1-16	13-26
X	US 2003/180272 A1 (ISOLAURI ERIKA ET AL) 25 September 2003 (2003-09-25)	1-12
Y	page 1, column 1 - page 2, column 1	13-26
Y	WO 03/071883 A (CENTRO SPERIMENTALE DEL LATTE S.P.A; BIANCHI SALVADORI, BRUNA; PIROVAN) 4 September 2003 (2003-09-04)	13-26
	page 6 - page 9; claims 1-22	
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☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

* & * document member of the same patent family

Date of the actual completion of the international search

6 February 2006

Date of mailing of the international search report

16/02/2006

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Markopoulos, E

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2005/003214

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TIMMERMAN H M ET AL: "Monostrain, multistrain and multispecies probiotics-A comparison of functionality and efficacy" INTERNATIONAL JOURNAL OF FOOD MICROBIOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 96, no. 3, 15 November 2004 (2004-11-15), pages 219-233, XP004580747 ISSN: 0168-1605 page 220, column 1 - page 221, column 1, paragraph 1; table 7 page 231	1-26
Y	WO 01/97821 A (MUCOPROTEC PTY LTD; CLANCY, ROBERT; PANG, GERALD; BORODY, THOMAS; DUNK) 27 December 2001 (2001-12-27) page 2 - page 6	1-26

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2005/003214

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 20202562	U1	23-05-2002	NONE
US 2003180272	A1	25-09-2003	AU 7257201 A 02-01-2002
			DE 01951716 T1 23-06-2005
			EP 1296694 A1 02-04-2003
			WO 0197822 A1 27-12-2001
			FI 20001460 A 21-12-2001
			US 2006013806 A1 19-01-2006
WO 03071883	A	04-09-2003	AU 2003212265 A1 09-09-2003
			EP 1478246 A1 24-11-2004
			IT MI20020399 A1 28-08-2003
WO 0197821	A	27-12-2001	CN 1444484 A 24-09-2003
			EP 1296693 A1 02-04-2003
			JP 2003535903 T 02-12-2003
			US 2003180260 A1 25-09-2003