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(54) Title: PROBIOTICA AND POLYPHENOL

(57) Abstract: The present invention relates to the field of infection caused by undesirable bacteria and associated diseases, and in particular to compositions comprising probiotica and polyphenols, which have the capacity to prevent and/or treat these infections and that may also be used as a preservative.



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PROBIOTICA AND POLYPHENOL

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Field of the invention

The present invention relates to the field of infection caused among others by Gram positive Bacteria and associated diseases, and in particular to compositions comprising probiotica and polyphenol, which have the capacity to prevent and/or treat these infections and that may also be used as a preservative.

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Background of the invention

Microbial pathogens remain a serious threat to human health despite the availability of traditional anti-microbial therapies. Such pathogens are known to be both Gram positive and Gram negative bacteria. *Staphylococci* are a group of Gram positive bacteria that are known to cause numerous infections, including neonatal infections, mastitis, post-operative infections, furuncles and carbuncles, pneumonia, bacteremia, endocarditis and enterocolitis (1). Pathogenic *streptococci*, including *Streptococcus pyrogenes* are commonly associated with the skin and nasopharynx where they may cause disease. *Streptococcus pneumoniae* is another human pathogen that causes infection in the lower respiratory tract (2). Other *streptococci*, such as *Streptococcus mutans*, are involved in plaque formation on teeth and therefore are of great concern in relation to dental health. Gram positive bacteria such as *staphylococci* and to some extent *streptococci* often carry multiple antibiotic resistances, which makes successful treatment difficult and in some cases impossible. Certain Gram negative bacteria are also known to cause prolonged and even chronic infections due to a lack of effective treatments. *Helicobacter pylori* is an example on such an pathogen. Gastric infection by this pathogen would mostly lead to peptic ulcer, in some cases (1%) developing to gastric cancer. Gastric infection with *H. pylori* is often treated with a combination therapy. However, emerging strains with resistance to such therapies are a problem (3). Due to the demand for new effective treatments, alternative treatment methods have been explored. Such methods include the use of probiotics for treatment of infections of the

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gastrointestinal tract (4-6) the nasopharynx (7) and the skin (8). Epigallocatechin gallate (EGCG) is a compound that provides some protection against infection by *Helicobacter pylori* (9) and inhibits many other pathogenic bacteria in vitro. High dosage levels of EGCG may be required to achieve desired inhibition pathogens in vivo.

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In addition to a direct effect on growth of pathogens, it is known that on one side probiotics and on the other side EGCG have immune modulatory properties (10, 11), which also may contribute to the treatment of infections by certain pathogens.

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For a probiotic culture to be active, it needs to compete with other microorganisms, in the targeted area. Therefore, prebiotic compounds have been useful in stimulating growth and activity of probiotics in specific targeted areas to maximize the beneficial effect of probiotic cultures. The drawback with such prebiotics is that they may also stimulate growth and activity of certain undesirable bacteria.

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There is still a need for improved treatments of infections caused by bacteria, which do not have the drawback of the known treatments (among other emergence of resistant strains).

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Detailed description of the invention

The invention relates to a composition comprising a probiotic and a polyphenol.

A probiotic is defined herein as a live microbial strain, which beneficially affects the human host cell by improving its microbial balance. Preferably, the probiotic is selected from the group consisting of a *Lactobacillus*, a *Bifidobacterium* species and mixture thereof.

According to a more preferred embodiment, the *Lactobacillus* is selected from the group consisting of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus helveticus*, *Lactobacillus fermentum* and mixtures thereof and the *Bifidobacterium* is selected from the group consisting of *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium lactis*, *Bifidobacterium animalis* and mixture thereof.

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Even more preferably, the probiotica is selected from the group consisting of the *Lactobacillus acidophilus* CBS 116411, the *Lactobacillus casei* CBS 116412, and the *Bifidobacterium lactis* B94 CBS 118529 all three protected by the trademark LAFTITM (DSM, The Netherlands), a *Bifidobacterium* species. According to a preferred embodiment, the probiotic used in the composition of the invention comprises a biologically pure culture of said deposited strain. According to a more preferred embodiment, the probiotic used in the composition of the invention comprises a biologically pure culture of said deposited strain in combination with any other valuable probiotic strain.

The polyphenol present in the composition of the invention derives preferably from a tea extract or is preferably a tea extract. According to a more preferred embodiment, the tea extract is a green tea extract. According to a preferred embodiment, the polyphenol is or comprises catechins. The major catechins present in green tea extract are epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC) and epicatechin (EC). Preferably, the green tea extract comprises EGCG. EGCG is a polyphenol derived from the green tea plant *Camellia sinensis*. EGCG preparations are already known. A process for preparing EGCG preparations has already been described for example in EP 1,077,211. In this process, EGCG is highly enriched starting from any green tea extract, e.g. green tea leaves are typically extracted with hot or cold water to form a solution containing tea catechins and caffeine. This green tea solution can be further concentrated to form either a concentrated extract solution or a dry powder. Tea extract powders are commercially available e.g. from Guizhou Highyin Biological Product Co., Guiyang, P. R. China, or Zhejiang Zhongke Plant Technical Co. Ltd., Hangzhou, Zhejiang, P. R. China. More preferably, the green tea extract comprises as major compound EGCG. Major compound means that the green tea extract comprises at least 90% w/w, preferably at least 93 %, more preferably at least 95 % EGCG. Most preferably, the green tea extract is TeavigoTM (DSM Nutritional products Ltd., Kaiseraugst, Switzerland) or is EGCG.

According to a most preferred embodiment, the composition comprises EGCG and a probiotica is selected from the group consisting of the *Lactobacillus acidophilus*

CBS 116411, the *Lactobacillus casei* CBS 116412, and the *Bifidobacterium lactis* B94 CBS 118529

Green tea extract is a powerful antioxidant and is known to have some anti-microbial activities, against undesirable bacteria such as Gram positive and Gram negative bacteria. Gram positive bacteria appear to be particularly sensitive to polyphenol. Surprisingly, we demonstrate that several probiotic strains (*Lactobacilli* and *Bifidobacteria* strains), which are Gram positive bacteria are relatively resistant to the anti-microbial activity of green tea extract. These strains grow at concentrations that are close to 10 times higher than the minimum concentrations that inhibit other Gram positive bacteria and some Gram negative bacteria. Therefore, the composition of the invention could be used in any types of applications wherein the undesirable agent is among others a Gram positive bacteria: the green tea extract would act as an anti-microbial agent which would allow the probiotic to colonize the treated human, animal or product. Increased colonization capacity and activity of the probiotic will improve the performance and efficacy of the probiotic culture. It has been surprisingly found that the combination of these two components together (polyphenol and probiotic) works synergistically such that the combination is more effective against undesirable bacteria than each agent taken separately. Surprisingly, the composition of the invention has a synergistic action on the modulation of the immune system.

The composition of the invention has the capacity to kill an undesirable bacterium or to inhibit its growth, or to prevent the germination of its spores *in vitro* in a given medium and/or *in vivo* in a given food product matrix, crop protection application, pharmaceutical or cosmetic product. Undesirable bacteria are defined as being either a Gram positive or a Gram-negative bacterium. Preferably, undesirable bacteria are bacteria, which are not defined as probiotic. Undesirable Gram-negative bacteria that are selectively targeted by the composition of the invention are a.o. *Helicobacter pylori*. Preferably, undesirable Gram-positive bacteria that are susceptible and are targeted by the composition of the invention are selected from the group consisting of a *Streptococcus*, *Staphylococcus* species and mixture thereof. The effect of killing undesirable bacteria or inhibiting their growth is preferably measured using the following *in vitro* assay: 10^7 cfu (colony forming units) of undesirable bacteria such as *Streptococcus pyrogenes* or *Staphylococcus aureus* (as used in the example) are inoculated into a test media containing 10^7 cfu of probiotic *Lactobacillus acidophilus* L10 CBS 116411 and/or *Lactobacillus casei* L26 CBS

116412 and/or *Bifidobacterium lactis* B94 CBS 118529 (all three strains being protected by the trademark LAFTI™) with 100 µg/ml Teavigo™. The following two experiments serve as controls: (1) 10⁷ cfu of the chosen undesirable bacteria as defined above are inoculated into a test media containing 10⁷ cfu of one of the probiotic LAFTI™ as defined above and (2) 10⁷ cfu of the chosen undesirable bacteria is inoculated into a test media containing 100 µg Teavigo™ /ml. The relative inhibition of undesirable bacteria, in comparison to the controls, is monitored for at least 4h. The number of cfu is preferably estimated by enumeration of undesirable bacteria on Nutrient agar (Oxoid, UK), after incubation of inoculated agar plates at 37°C for at least 48 hours under anaerobic or microaerophilic conditions. According to a preferred embodiment, the composition is said to have the capacity to kill undesirable bacteria if the reduction that is at least 20% greater than in the above experiments defined as control experiment 1 and 2. Preferably, at least 1 additional log₁₀ unit of the undesirable bacteria have been killed, more preferably at least 2 log₁₀ units, even more preferably at least 3 log₁₀ units, even more preferably at least 4 log₁₀ units, even more preferably at least 5 log₁₀ units, even more preferably at least 6 log₁₀ units and most preferably at least 7 log₁₀ units more than the reductions achieved in the control experiments 1 or 2.

According to another preferred embodiment, the composition is said to have to prevent the growth of the undesirable bacteria if in the *in vitro* test defined above the number of undesirable bacteria at the end of the assays is the same as at the beginning of the assay. Preferably, the number of undesirable bacteria at the end of the assays is at least 10% less than the number of undesirable bacteria compared to control experiment 1 or 2, more preferably at least 1 log₁₀ unit less, even more preferably at least 2 log₁₀ unit less, even more preferably at least 50% less and most preferably at least 7 log₁₀ units less.

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Depending on the type of applications and/or administration, the composition of the invention may comprise:

- at least 10⁶ cfu probiotic, or at least 10⁷ cfu probiotic, at least 10⁸ cfu probiotic or at least 10⁹ cfu probiotic or at least 10¹⁰ cfu probiotic or at least 10¹¹ cfu probiotic associated with
- at least 100µg/ml polyphenol or EGCG, or at least 200 µg/ml polyphenol or EGCG, or at least 300 µg/ml polyphenol or EGCG, or at least 50 mg/ml polyphenol or

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EGCG, or at least 100 mg/ml polyphenol or EGCG or at least 300 mg/ml polyphenol or EGCG or at least 10 mg polyphenol or EGCG/capsule or tablet, or at least 100 mg polyphenol or EGCG/capsule or tablet or at least 500 mg polyphenol or EGCG/capsule or tablet, or at least 1000 mg polyphenol or EGCG/capsule or tablet.

The preferred probiotic strains have been defined earlier in the description. The two components (probiotic, polyphenol) may be administered sequentially to the human to be treated or to the product to be treated. In this case, the composition of the invention may be manufactured as a kit comprising two separate constituents, the probiotic and the polyphenol. Alternatively, the two components may be first formulated together and added as a single component to the human or to the product to be treated. Depending on the type of application of the composition of the invention, preferred formulations are given later on in the description.

According to another preferred embodiment, the composition of the invention comprises as probiotic any of the strains as defined earlier, preferably the strain *Lactobacillus acidophilus* CBS 116411 and/or the strain *Lactobacillus casei* CBS 116412 and/or *Bifidobacterium lactis* CBS 118529 and mixtures thereof with polyphenol or EGCG or preferably TeavigoTM in combination with at least one of the following ingredients in order to get a synergistic combination on the human being treated. Preferred ingredients are: vitamin A, vitamin C, vitamin D3, vitamin E, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin C, nicotinamide, folic acid, beta carotene, lutein, Zinc, Copper, selenium, and prebiotic.

According to a more preferred embodiment, the composition of the invention comprises a probiotic, a polyphenol and a prebiotic. It has been surprisingly found that the combination of these three components together (polyphenol, probiotic and prebiotic) works synergistically such that the combination is more effective against undesirable bacteria than each agent taken separately. Surprisingly, the composition of the invention has a synergistic action on the modulation of the immune system.

Prebiotic is defined as a component, which has a favourable influence on the growth of certain beneficial intestinal bacteria, preferably probiotic. Preferably the prebiotic is selected from the group consisting of pentosans (or arabinoxylans), β glucans, pectins, and pectic polysaccharides, mannans, arabinans, galactans, fructo-

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oligosaccharides, resistant starch and mixture thereof. Prebiotic have been fully described in US 6,241,983. Most preferred fructo-oligosaccharides are selected among the group consisting of inulin, banana fiber, and soy fructo-oligosaccharides or soy oligosaccharides. Most preferred mannans are selected among the group consisting of guar gum, locust bean gum, konjac and ad xanthan gum. Resistant starch as used in the context of this invention includes those forms defined as RS1, RS2, RS3, and RS4 as defined in Brown et al (Brown, Mc Knaught and Moloney (1995) Food Australia 47:272-275). Resistant starch and modified resistant starch have been both extensively described in US 6,348,452.

The Composition of the invention is preferably for use as a medicament. According to a further aspect, the invention relates to the use of the composition of the invention for the preparation of a medicament for preventing and/or treating infections caused by undesirable bacteria and associated diseases in a human being in a need thereof. The medicament, which comprises the composition of the invention is preferably used for preventing and/or treating infections caused by undesirable bacteria and associated diseases in a human being in a need thereof. Preferably, the undesirable bacterium is among other a Gram positive bacteria. In the context of this invention, a human being in the need of the composition of the invention is preferably defined as follows: human subjects that experience some form of fatigue and/or stress, bed sores, gingivitis, nose, throat infection and peptic ulcer. Stress and fatigue can have any origin but could have a professional origin: shiftwork, international travel, academic stress in students, over-training in sport (athletes). Alternatively and/or in combination with stress having a professional origin, stress could also have a domestic origin: family stress, stress caused by children. Alternatively and/or in combination with previous mentioned possible origins of stress, stress is caused by mid-life crisis and/or diet-related stress. More preferably, the stress and/or fatigue cause impaired physical performance in human over-training in sport such as athletes. Humans, in a need of the composition of the invention are preferably individuals that have been subjected to physical stress, e.g. athletes or other individuals that are susceptible to infections caused by among other Gram-positive bacteria

According to a preferred embodiment, preventing and/or treating infections caused by undesirable bacteria in human means that after daily oral administration of at

least 10^6 cfu (colony forming units) of probiotic associated with at least 10mg polyphenol per day during at least 1 day, a normal level of at least one of the following biomarkers is detected using a specific given assay. According to a preferred embodiment, the biomarker is nitric oxide (NO), which is measured by a standard method known to the skilled person such as the Griess reaction (14). A normal level of NO is 150-200 μ M using this reaction. According to another preferred embodiment, the biomarker is the level of presence of an undesirable bacterium for example in the nose. The undesirable bacterium is preferably a *Streptococcus* or a *Staphylococcus* species. The undesirable bacterium is detected by Nutrient agar. According to another preferred embodiment, the biomarker is the Cross Reactive Protein (CRP), which is measured as described by (15). A normal level of CRP is less than 7mg/L blood as measured by this assay. More preferably, the treatment is continued during at least 5 days to obtain this effect, even more preferably during at least 10 days, even more preferably during at least 30 days, even more preferably at least 60 days, even more preferably at least four month and most preferably during at least one year.

In particular, the composition of the invention can be used to prevent and/or treat gingivitis (mouthwash), skin infection (skin cream), nose and throat infections (tablet, capsule, spray), *Helicobacter* infections (tablet, capsule). The skilled person will understand that depending on the type of infections treated and targeted area, the type of formulation and possibly the quantity of each constituent of the composition of the invention would have possibly to be adjusted to obtain the most efficient formulation.

The composition of invention may be in any galenic form that is suitable for administrating to the animal body including the human body, especially in any form that is conventional for oral administration, e.g. in solid form such as (additives/supplements for) food or feed, food or feed premix, fortified food or feed, tablets, pills, granules, dragées, capsules, and effervescent formulations such as powders and tablets, or in liquid form such as solutions, emulsions or suspensions as e.g. beverages, pastes and oily suspensions. The pastes may be filled into hard or soft shell capsules, whereby the capsules feature e.g. a matrix of plant proteins or ligninsulfonate for example. Examples for other application forms are forms for topical, transdermal, parenteral or injectable administration. The composition of the invention may be in the form of controlled (delayed) release formulations.

Preferably, for topical applications (skin) or mouthwash, the composition comprises:

- at least 100 µg/ml polyphenol or EGCG or 200 µg/ml polyphenol or EGCG or at least 300 µg/ml polyphenol or EGCG associated with
- at least 10⁶ cfu/ml probiotic or at least 10⁷ cfu/ml probiotic or at least 10⁸ cfu/ml probiotic.

5 Preferably, for oral administration, the preparation comprises:

- at least 10 mg polyphenol or EGCG as the daily dose, or at least 200 mg polyphenol or EGCG as the daily dose or at least 500mg polyphenol or EGCG as the daily dose, or at least 1000 mg polyphenol or EGCG as the daily dose associated with
- 10 - at least 10⁸ cfu probiotic or at least 10¹⁰ cfu probiotic at least 10¹¹ cfu probiotic.

Preferably, for both types of applications, the polyphenol is as defined earlier a green tea extract or derived thereof or EGCG or TEAVIGO™. Preferably, the probiotic is one of the probiotic strain defined earlier on.

The invention further relates to a product comprising the composition of the invention.

15 The product is preferably selected from the group consisting of a medicament, a feed product, a food product, a cosmetic, an oral hygiene product and an agricultural product.

In a preferred embodiment, the composition of the invention is a medicament or for use in the preparation of a medicament or is a food product or part thereof. According to a preferred embodiment, the composition of the invention is used for the manufacture of said medicament or food product. The medicament of the invention may comprise any physiologically or pharmacologically acceptable excipient and/or diluent. If the composition of the invention is part of a food product, the composition of the invention can be present as a dry formulation, e.g. granule, powder, tablet or the like. Alternatively, the composition of the invention may be formulated in a liquid form. The food product of the invention comprising the composition of the invention may comprise any physiologically or acceptable excipient and/or diluent. Preferred food products are cultured milk, yoghurt, cheese, milk drink, milk powder, sport drink, coveratures (defined as mixtures of oil, sugar(s) and milk protein (whey)), infant formulas and fermented meat products.

30 Since the composition of the invention may be incorporated into a (food) product, which already contains endogenous probiotic and/or polyphenol, all amounts of probiotic and/or polyphenol given in the description relates to added amounts of these components.

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Alternatively and according to another preferred aspect of the invention, a *Lactobacillus* strain as defined before, preferably the *Lactobacillus acidophilus* strain CBS 116411 and/or the *Lactobacillus casei* CBS 116412 and/or *Bifidobacterium lactis* CBS 118529 and mixtures thereof and a polyphenol, preferably EGCG as defined before
5 are used in the preparation of a food product for preventing and/or combating infections associated with undesirable bacteria and associated diseases in a human being in a need thereof. Preferably, the undesirable bacteria is a Gram positive bacteria as defined before.

10 The product (medicament or food product) of the invention comprises a probiotic, preferably a *Lactobacillus* strain as defined earlier, preferably the *Lactobacillus acidophilus* strain CBS 116411 and/or the *Lactobacillus casei* CBS 116412 and/or *Bifidobacterium lactis* CBS 118529 and a polyphenol, preferably EGCG as defined before in an effective amount to prevent and or treat infections associated with undesirable
15 bacteria and associated diseases. According to a preferred embodiment, the food product or medicament comprises at least 10^6 to 10^8 cfu probiotic per gram product and at least 2 to 5 mg polyphenol or EGCG per gram product. According to a more preferred embodiment, the food product or medicament comprises at least 10^6 to 10^8 cfu probiotic and at least 2 to 5 mg TEAVIGO™ or EGCG per gram product.

20 According to another preferred embodiment, the composition and the product of the invention (medicament and food product) is in the form of tablet, capsule, powder or granule, orally liquid administered liquid preparations, or suppositories, or dry preparations. More preferably, the probiotic present in the composition and the product of the invention is in the form of an enteric tablet, capsule, powder or granule, meaning it will
25 survive the stomach and arrive intact in the intestine. All these forms can be prepared by known means, using food grade, respectively pharmaceutically acceptable carriers, excipients, solvents or adjuvants. For the preparation of the medicament, standard ingredients and method of preparation as already described in Remington: The science and practice of pharmacy, 1995, Mack Publishing, Co Easton, PA 18042, USA can be
30 used, which content is herewith incorporated by reference.

The invention therefore also relates to a method wherein the composition of the invention, comprising a probiotic and a polyphenol preferably EGCG and/or preferably a

Lactobacillus strain as defined earlier, more preferably the *Lactobacillus acidophilus* strain CBS 116411 and/or the *Lactobacillus casei* CBS 116412 and/or *Bifidobacterium lactis* CBS 118529 are formulated in a food product or in a medicament, and said food product or medicament is administered to a given human being in an amount effective for preventing and/or treating diseases associated with undesirable bacterial infections. Therefore, the invention further relates to a method for preventing and/or treating infections associated with undesirable bacteria, preferably Gram positive bacteria and associated diseases in a given human being. This method comprises the step of administering the medicament or food product of the invention to said human being. It will be appreciated that the exact dosage of both essential active ingredients which are the probiotic and the polyphenol, constituting an amount effective for preventing and/or treating infections associated with undesirable bacteria and associated diseases in a human being in a need thereof may vary depending on the specific nature of the clinical conditions being treated, severity of the condition, age of human being treated, weight and condition of the human being treated, mode of administration of the dosage form, and the specific formulation being administered.

Preferably, administering the composition or the product of the invention preferably means ingesting said product to give a daily dose of at least 10^8 cfu probiotic and 10 mg polyphenol or EGCG per day. More preferably, an amount of the product that corresponds to 10^{10} cfu probiotic and 800 mg polyphenol or EGCG per day.

The composition of the invention due to its anti-bacterial activity can act as a preservative and may be used as such. The preservative composition of the invention may additionally comprise an anti-fungal agent selected from the group consisting of a weak acid preservative, a polyene anti-fungal compound, sulphur dioxide, sulphites, nitrate, nitrite, dimethyl dicarbonate, biphenyl, diphenyl, orthophenylphenol, thiobendazole, an inorganic acid, an imidazole and mixture thereof. The different anti-fungal agents that may be present in the composition of the invention are below further defined:

1. a weak acid preservative such as sorbic acid, propionic acid, benzoic acid, a p-hydroxybenzoic acids, lactic acid, citric acid, acetic acid or an alkali metal or alkali earth metal salt thereof;
2. a polyene anti-fungal compound, preferably natamycin;

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3. sulphur dioxide or sulphites;
4. nitrate and nitrite;
5. dimethyl dicarbonate;
6. biphenyl, diphenyl, orthophenylphenol or thiobendazole ;
- 5 7. an inorganic acid, such as hydrochloric acid;
8. an imidazole such as imazalil; and/or
9. any anti-fungal compound known in the art for use as a preservative for food products, crop protection or after-harvest treatment of fruits, vegetables or cereals, pharmaceutical or cosmetic products.

10 The present invention further relates to the use of the composition of the invention comprising a probiotic and a polyphenol for killing among other undesirable bacteria such as Gram-positive bacteria or for inhibiting their growth or for preventing the germination of their spores. The composition of the invention can be used for the preparation of any types of products such as a food product or a medicament for
15 preventing and/or treating infections caused by undesirable bacteria and associated diseases in a human being in a need thereof. The Gram-positive bacteria, the human being in need of the composition and the type of food products are as defined above including the preferences as described. The composition of the invention can be used as a preservative for the preparation of a product.

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The invention will now be illustrated by the following example, which should not be construed as limiting the scope of the invention.

Examples

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General experimental conditions

In vitro experiments were performed to assess the effect of TEAVIGO™ on microbial growth of the probiotic strains *L. acidophilus* LAFTI® L10 (CBS 116411), *B. lactis* LAFTI® B94 (CBS 118529) and *L. casei* LAFTI® L26 (CBS 116412), as well as the pathogenic bacteria, *S. aureus* ATCC 25923 and *S. pyogenes* ATCC 10389.

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The objectives of this study were to (i) assess the antimicrobial activity of TEAVIGO™ against pathogenic bacteria, *Staphylococcus aureus* and *Streptococcus pyogenes* in liquid medium, (ii) assess the effect of TEAVIGO™ on growth of probiotic strains *Lactobacillus acidophilus* LAFTI® L10, *Bifidobacteria lactis* LAFTI® B94 and *Lactobacillus casei* LAFTI® L26 in liquid medium and (iii) explore the possibility of combined therapy for infectious diseases using TEAVIGO™ and probiotics together.

Cultures and media

The strains *S. aureus* ATCC 25923 and *S. Pyogenes* ATCC 10389 were obtained from the UNSW Microbiology Culture Collection. Three probiotic strains, *L. acidophilus* LAFTI® L10 (CBS 116411), *B. lactis* LAFTI® B94 (CBS 118529) and *L. casei* LAFTI® L26 (CBS 116412) were supplied by DSM Food Specialties, Australia.

S. aureus was grown on nutrient agar medium under aerobic conditions. *L. acidophilus*, *B. lactis* and *L. casei* were grown in anaerobic jars under CO₂ + N₂ atmosphere (AnaroGen™, Oxoid) at 37 °C on reinforced clostridial medium (RCM) agar media. *S. pyogenes* was grown on blood agar media under microaerophilic conditions. The susceptibility tests were carried out in RCM broth. All media were purchased from Oxoid, England.

Example 1: *In vitro* growth responses of probiotic strains and pathogenic bacteria to TEAVIGO™

Antimicrobial effect and MIC of TEAVIGO™ in liquid medium

TEAVIGO™ was obtained from DSM Nutritional Products Ltd. Kaiseraugst Switzerland. TEAVIGO™ is a green tea extract and contains minimum 90% of EGCG, a tea polyphenol. TEAVIGO™ was dissolved at a concentration of 10 mg /ml in milli-Q water, and was sterilised by passing through a 0.45 µm filter. The minimum inhibitory concentrations (MICs) of TEAVIGO™ were determined by the broth microdilution method, at an inoculum of about 1×10^7 cfu per well. After incubation at 37 °C for 24 h, all of the wells were examined macroscopically for bacterial growth, and the lowest concentration of two fold serially diluted TEAVIGO™ at which no visible growth occurred was defined as its MIC

Anti-microbial activity of TEAVIGO™ in liquid medium

The anti-microbial effect of TEAVIGO™ was assessed using seven bacterial strains in RCM broth containing 0, 25, 50, 100, 200, 400 and 800 µg/ml TEAVIGO™.

5 Visible growth of bacteria is illustrated in table 1.

Table 1. Visible growth (+) of bacteria in liquid medium containing various concentration of TEAVIGO™

Test strain	Concentration of TEAVIGO™ (µg /ml)						
	800	400	200	100	50	25	0
<i>S. aureus</i> ATCC 25923	-	-	-	±	+	+	+
<i>S. pyogenes</i> ATCC 10389	-	-	-	±	+	+	+
<i>L. acidophilus</i> L10	+	+	+	+	+	+	+
<i>B. lactis</i> B94	+	+	+	+	+	+	+
<i>L. casei</i> L26	+	+	+	+	+	+	+

Legends:

- 10 +: visible growth of bacteria
 -: no visible growth of bacteria

Medium: reinforced clostridial medium (RCM) broth. All experiments were performed in triplicate in two separate experiments.

15

Minimum inhibitory concentrations of TEAVIGO™ on seven bacteria

MICs were calculated from Table 1. As shown in table 2, the seven tested strains showed quite different susceptibility to TEAVIGO™. Compared to the MICs of TEAVIGO™ against *S. aureus* and *S. pyogenes* (100-200 µg/ml), the MICs of
 20 TEAVIGO™ against probiotic LAFTI® strains were higher than 800 µg/ml.

Table 2. Comparison of TEAVIGO™ MICs against four strains of pathogenic bacteria and three probiotic LAFTI® strains

<i>Pathogenic bacteria</i>		<i>Probiotic strains</i>	
<i>Test strain</i>	<i>MIC (µg/ml)</i>	<i>Test strain</i>	<i>MIC (µg/ml)</i>
<i>Gram-positive bacteria</i>		<i>Gram-positive bacteria</i>	
<i>S. aureus ATCC 25923</i>	100-200	<i>L. acidophilus L10</i>	>800
<i>S. pyogenes ATCC 10389</i>	100-200	<i>B. lactis B94</i>	>800
		<i>L. casei L26</i>	>800

5 MIC, minimum inhibitory concentration. All experiments were performed in triplicate in two separate experiments.

10 It was recently reported that gram-positive bacteria generally are more sensitive to EGCG than gram-negative bacteria (12). However our results indicated that LAFTI® probiotic strains, which are also gram-positive, demonstrated lower susceptibility to TEAVIGO™. Comparable result reported that EGCG shows bactericidal activities against gram-negative bacteria *Vibrio* (13). Although EGCG reportedly have selective bactericidal activity against pathogenic bacteria, no significant difference was observed in the MICs against bacterial phenotypes (13).

15

Conclusion

20 The minimum inhibitory concentrations (MICs) of TEAVIGO™ against *S. aureus* and *S. pyogenes* (100-200 µg/ml) were much lower than the values against the probiotic strains tested (>800 µg/ml). These results demonstrate the potential for combined therapy using TEAVIGO™ and probiotics on microbial infection caused by *S. aureus* and *S. pyogenes* etc., since probiotics and chemicals from natural products are two successful strategies as adjuncts or alternative to conventional antibiotic therapy.

Example 2: Synergistic effect of Teavigo™ and probiotics on pathogenic bacteria

The probiotic strains and pathogenic strains defined in general experimental conditions were used in this example and cultivated the same way as defined in general experimental conditions and in example 1 unless otherwise indicated.

In the following tables, the synergistic effect of Teavigo™ associated with either *Lactobacillus acidophilus* L10, *Lactobacillus casei* L26 or *Bifidobacterium lactis* B94 was demonstrated.

Table 3. Synergistic effect of Teavigo™ and *Lactobacillus acidophilus* L10 on the growth of *Staph. aureus* at 4h and 24 h.

Concentration of Teavigo* (µg/ml)	<i>Staph. aureus</i> ** was inoculated at:							
	5%				0.5%			
	4 h ***		24 h		4 h		24 h	
	-	+ L10	-	+	-	+	-	+
0	7×10^5	7.5×10^4	5×10^7	ND	2.1×10^4	1.3×10^4	1×10^7	ND
100	4.5×10^4	3.0×10^4	2×10^5	ND	5×10^2	ND	1×10^3	ND
200	2.8×10^4	1.6×10^4	1×10^4	ND	ND	ND	ND	ND
400	2.5×10^4	1.2×10^4	ND	ND	ND	ND	ND	ND

Legends:

* RCM broth (fermentation broth) without Teavigo™ (0) or with different concentrations of Teavigo™ (100, 200, 400 µg/ml)

** *Staph. aureus* was inoculated at (0 h) at a 5% concentration (inoculation ratio to L10: 1:1), or at 0.5% (inoculation ratio to L10: 0.1:1)

*** Fermentation was carried out anaerobically at 37°C for 4 h or 24 h

+: with L10, L10 was inoculated at 0 h at 5% (v/v)

- : without L10

ND: not detected

The results (viable count of *Staph. aureus*) indicate that at 4 h the growth of *Staph. aureus* was significantly reduced by the synergistic effect of Teavigo™ and L10. At 24 h, the growth of *Staph. Aureus* was completely inhibited by the synergistic effect.

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Table 4: The effect of Teavigo™ (100-400 µg/ml) and *Staph. aureus* (0.1:1, 1:1 inoculation ratio) on the growth of *Lactobacillus acidophilus* L10.

Lactobacillus acidophilus L10 was inoculated at 0 h at 5% (v/v). For other conditions see legends of Table 3.

Concentration of Teavigo (µg/ml)	<i>Staph. aureus</i> was inoculated at 0 h at:					
	0		0.5%		5%	
	4 h	24 h	4 h	24 h	4 h	24 h
0	6.9×10^6	5×10^7	4.9×10^6	1×10^8	5.6×10^6	1×10^8
100	7×10^6	7×10^7	4×10^6	5×10^7	5.7×10^6	5×10^7
200	4.9×10^6	5×10^7	3.7×10^6	8×10^7	5.2×10^6	6×10^7
400	4.0×10^6	3×10^7	3.5×10^6	4×10^7	4.5×10^6	2×10^7

The results (viable count of L10) demonstrate that neither Teavigo™ nor *Staph. aureus* had a significant effect on the growth of *Lactobacillus acidophilus* L10

Table 5. Synergistic effect of Teavigo™ and *Bifidobacterium lactis* B94 on the growth of *Staph. aureus* at 4h and 24 h.

Concentration of Teavigo* (µg/ml)	<i>Staph. aureus</i> ** was inoculated at:							
	5%				0.5%			
	4 h ***		24 h		4 h		24 h	
	-	+ B94	-	+	-	+	-	+
0	7×10^5	2.4×10^4	5×10^7	ND	2.1×10^4	9×10^3	1×10^7	ND
100	4.5×10^4	2×10^4	2×10^5	ND	5×10^2	ND	1×10^3	ND
200	2.8×10^4	1.5×10^4	1×10^4	ND	ND	ND	ND	ND
400	2.5×10^4	1×10^4	ND	ND	ND	ND	ND	ND

5 Legends:

* RCM broth (fermentation broth) without Teavigo™ (0) or with different concentrations of Teavigo™ (100, 200, 400 µg/ml)

** *Staph. aureus* was inoculated at (0 h) at a 5% concentration (inoculation ratio to B94: 1:1), or at 0.5% (inoculation ratio to B94: 0.1:1)

10 *** Fermentation was carried out anaerobically at 37°C for 4 h or 24 h

+: with B94, B94 was inoculated at 0 h at 5% (v/v)

- : without B94

ND: not detected

15 The results (viable count of *Staph. aureus*) indicate that at 4 h the growth of *Staph. aureus* was significantly reduced by the synergistic effect of Teavigo™ and *Bifidobacterium lactis* B94. At 24 h, the growth was completely inhibited by the synergistic effect.

Table 6. The effect of TeavigoTM (100-400 µg/ml) and *Staph. aureus* (0.1:1, 1:1 inoculation ratio) on the growth of *Bifidobacterium lactis* B94.

Bifidobacterium lactis B94 was inoculated at 0 h at 5% (v/v). For other conditions see legends of Table 3.

Concentration of Teavigo (µg/ml)	<i>Staph. aureus</i> was inoculated at 0 h at:					
	0		0.5%		5%	
	4 h	24 h	4 h	24 h	4 h	24 h
0	2.5×10^8	3.8×10^8	2.9×10^8	3.9×10^8	2.6×10^8	3.8×10^8
100	2.6×10^8	3.8×10^8	2.2×10^8	3.8×10^8	2.6×10^8	3.7×10^8
200	2.2×10^8	3.7×10^8	1.7×10^8	3.9×10^8	2.2×10^8	3.8×10^8
400	1.6×10^8	1.8×10^8	2.1×10^8	1.7×10^8	1.6×10^8	1.6×10^8

The results (viable count of *Bifidobacterium lactis* B94) demonstrate that neither TeavigoTM nor *Staph. aureus* had a significant effect on the growth of *Bifidobacterium lactis* B94.

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Table 7. Synergistic effect of Teavigo™ and *Lactobacillus casei* L26 on the growth of *Staph. aureus* at 4h and 24 h.

Concentration of Teavigo* (µg/ml)	<i>Staph. aureus</i> ** was inoculated at:							
	5%				0.5%			
	4 h ***		24 h		4 h		24 h	
	-	+ L26	-	+	-	+	-	+
0	7×10^5	4×10^4	5×10^7	ND	2.1×10^4	8×10^3	1×10^7	ND
100	4.5×10^4	1.8×10^4	2×10^5	ND	5×10^2	ND	1×10^3	ND
200	2.8×10^4	1.1×10^4	1×10^4	ND	ND	ND	ND	ND
400	2.5×10^4	5×10^3	ND	ND	ND	ND	ND	ND

5

Legends

* RCM broth (fermentation broth) without Teavigo™ (0) or with different concentrations of Teavigo™ (100, 200, 400 µg/ml)

10 ** *Staph. aureus* was inoculated at (0 h) at a 5% concentration (inoculation ratio to L26: 1:1), or at 0.5% (inoculation ratio to L26: 0.1:1)

*** Fermentation was carried out anaerobically at 37°C for 4 h or 24 h

+: with L26, L26 was inoculated at 0 h at 5% (v/v)

- : without L26

ND: not detected

15

The results (viable count of *Staph. aureus*) indicate that at 4 h the growth of *Staph. aureus* was significantly reduced by the synergistic effect of Teavigo™ and *Lactobacillus casei* L26. At 24 h, the growth was completely inhibited by the synergistic effect.

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Table 8. The effect of Teavigo™ (100–400 µg/ml) and *Staph. aureus* (0.1:1, 1:1 inoculation ratio) on the growth of *Lactobacillus casei* L26.

Lactobacillus casei L26 was inoculated at 0 h at 5% (v/v). For other conditions see legends of Table 3.

Concentration of Teavigo (µg/ml)	<i>Staph. aureus</i> was inoculated at 0 h at:					
	0		0.5%		5%	
	4 h	24 h	4 h	24 h	4 h	24 h
0	7×10^7	2.8×10^8	1×10^8	2.4×10^8	9×10^7	2.9×10^8
100	1×10^8	3.0×10^8	1×10^8	2.8×10^8	1×10^8	2.8×10^8
200	8×10^7	2.1×10^8	8×10^7	2.2×10^8	1×10^8	2.3×10^8
400	9×10^7	1.8×10^8	1×10^8	1.3×10^8	6×10^7	1.9×10^8

The results (viable count of L26) demonstrate that neither Teavigo™ nor *Staph. aureus* had a significant effect on the growth of *Lactobacillus casei* L26.

Table 9. Synergistic effect of Teavigo™ and *Lactobacillus acidophilus* L10 on the growth of *Strep. pyogenes* at 4h and 24 h.

Concentration of Teavigo* (µg/ml)	<i>Strep. pyogenes</i> ** was inoculated at:							
	5%				0.5%			
	4 h ***		24 h		4 h		24 h	
	-	+ L10	-	+	-	+	-	+
0	2.7×10^6	2.1×10^6	6×10^7	ND	4×10^5	2×10^5	2×10^7	ND
100	1.3×10^6	1.1×10^6	8×10^3	ND	4×10^5	2×10^5	1.7×10^3	ND
200	2.5×10^6	1×10^6	5×10^3	ND	2×10^5	2×10^5	9×10^2	ND
400	1.3×10^6	1×10^6	9×10^2	ND	1×10^5	1×10^5	2×10^2	ND

Legends

5 * RCM broth (fermentation broth) without Teavigo™ (0) or with different concentrations of Teavigo™ (100, 200, 400 µg/ml)

** *Strep. pyogenes* was inoculated at (0 h) at a 5% concentration (inoculation ratio to L10: 1:1), or at 0.5% (inoculation ratio to L10: 0.1:1)

*** Fermentation was carried out anaerobically at 37°C for 4 h or 24 h

10 +: with L10, L10 was inoculated at 0 h at 5% (v/v)

- : without L10

ND: not detected

The results (viable count of *Strep. pyogenes*) indicate that at 4 h the growth of *Strep.*
 15 *pyogenes* was reduced by the synergistic effect of Teavigo™ and *Lactobacillus acidophilus* L10. At 24 h, the growth was completely inhibited by the synergistic effect.

Table 10. The effect of Teavigo™ (100-400 µg/ml) and *Strep. pyogenes* (0.1:1, 1:1 inoculation ratio) on the growth of *Lactobacillus acidophilus* L10

Lactobacillus acidophilus L10 was inoculated at 0 h at 5% (v/v). For other conditions see legends of Table 3.

Concentration of Teavigo (µg/ml)	<i>Strep. pyogenes</i> was inoculated at 0 h at:					
	0		0.5%		5%	
	4 h	24 h	4 h	24 h	4 h	24 h
0	4×10^7	1.1×10^8	3×10^7	6×10^7	2×10^7	7×10^7
100	3×10^7	5×10^7	2×10^7	8×10^7	3×10^7	6×10^7
200	3×10^7	7×10^7	3×10^7	8×10^7	2×10^7	6×10^7
400	2×10^7	3×10^7	2×10^7	3×10^7	3×10^7	4×10^7

The results (viable count of *Lactobacillus acidophilus* L10) demonstrate that neither Teavigo™ nor *Strep. pyogenes* had a significant effect on the growth of *Lactobacillus acidophilus* L10.

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Table 11. Synergistic effect of Teavigo™ and *Bifidobacterium lactis* B94 on the growth of *Strep. pyogenes* at 4h and 24 h.

Concentration of Teavigo* (µg/ml)	<i>Strep. pyogenes</i> ** was inoculated at:							
	5%				0.5%			
	4 h ***		24 h		4 h		24 h	
	-	+ B94	-	+	-	+	-	+
0	2.7×10^6	9×10^5	6×10^7	ND	4×10^5	2×10^5	2×10^7	ND
100	1.3×10^6	1.3×10^6	8×10^3	ND	4×10^5	1×10^5	1.7×10^3	ND
200	2.5×10^6	1.2×10^6	5×10^3	ND	2×10^5	2×10^5	9×10^2	ND
400	1.3×10^6	9×10^5	9×10^2	ND	1×10^5	5×10^4	2×10^2	ND

5

Legends

* RCM broth (fermentation broth) without Teavigo™ (0) or with different concentrations of Teavigo™ (100, 200, 400 µg/ml)

10 ** *Strep. pyogenes* was inoculated at (0 h) at a 5% concentration (inoculation ratio to B94: 1:1), or at 0.5% (inoculation ratio to B94: 0.1:1)

*** Fermentation was carried out anaerobically at 37°C for 4 h or 24 h

+: with B94, B94 was inoculated at 0 h at 5% (v/v)

- : without B94

ND: not detected

15

The results (viable count of *Strep. pyogenes*) indicate that at 4 h the growth of *Strep. pyogenes* was reduced by the synergistic effect of Teavigo™ and *Bifidobacterium lactis* B94. At 24 h, the growth was completely inhibited by the synergistic effect.

20

Table 12. The effect of Teavigo™ (100-400 µg/ml) and *Strep. pyogenes* (0.1:1, 1:1 inoculation ratio) on the growth of *Bifidobacterium lactis* B94.

B94 was inoculated at 0 h at 5% (v/v). For other conditions see Table 1a.

5

Concentration of Teavigo (µg/ml)	<i>Strep. pyogenes</i> was inoculated at 0 h at:					
	0		0.5%		5%	
	4 h	24 h	4 h	24 h	4 h	24 h
0	1×10^8	2.2×10^8	1.8×10^8	2.1×10^8	2.3×10^8	2.2×10^8
100	1.1×10^8	2×10^8	1.4×10^8	2×10^8	8×10^7	2×10^8
200	1×10^8	1.8×10^8	1×10^8	2.1×10^8	9×10^7	1.8×10^8
400	2×10^7	6×10^7	8×10^7	9×10^7	5×10^7	7×10^7

The results (viable count of *Bifidobacterium lactis* B94) demonstrate that neither Teavigo™ nor *Strep. pyogenes* had a significant effect on the growth of *Bifidobacterium lactis* B94.

Table 13. Synergistic effect of Teavigo™ and *Lactobacillus casei* L26 on the growth of *Strep. pyogenes* at 4h and 24 h.

Concentration of Teavigo* (µg/ml)	<i>Strep. pyogenes</i> ** was inoculated at:							
	5%				0.5%			
	4 h ***		24 h		4 h		24 h	
	-	+ L26	-	+	-	+	-	+
0	2.7×10^6	4×10^5	6×10^7	ND	4×10^5	2×10^5	2×10^7	ND
100	1.3×10^6	3×10^5	8×10^3	ND	4×10^5	2×10^5	1.7×10^3	ND
200	2.5×10^6	2×10^5	5×10^3	ND	2×10^5	2×10^5	9×10^2	ND
400	1.3×10^6	2×10^5	9×10^2	ND	1×10^5	1×10^5	2×10^2	ND

5

Legends

* RCM broth (fermentation broth) without Teavigo™ (0) or with different concentrations of Teavigo™ (100, 200, 400 µg/ml)

10 ** *Strep. pyogenes* was inoculated at (0 h) at a 5% concentration (inoculation ratio to L26: 1:1), or at 0.5% (inoculation ratio to L26: 0.1:1)

*** Fermentation was carried out anaerobically at 37°C for 4 h or 24 h

+: with L26, L26 was inoculated at 0 h at 5% (v/v)

- : without L26

ND: not detected

15

The results (viable count of *Strep. pyogenes*) indicate that at 4 h the growth of *Strep. pyogenes* was significantly reduced by the synergistic effect of Teavigo™ and *Lactobacillus casei* L26. At 24 h, the growth was completely inhibited by the synergistic effect.

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Table 14. The effect of Teavigo™ (100-400 µg/ml) and *Strep. pyogenes* (0.1:1, 1:1 inoculation ratio) on the growth of *Lactobacillus casei* L26.

Lactobacillus casei L26 was inoculated at 0 h at 5% (v/v). For other conditions see
5 Legends of Table 3.

Concentration of Teavigo (µg/ml)	<i>Strep. pyogenes</i> was inoculated at 0 h at:					
	0		0.5%		5%	
	4 h	24 h	4 h	24 h	4 h	24 h
0	1.4×10^8	3×10^8	1.8×10^8	3.1×10^8	1.3×10^8	3×10^8
100	1×10^8	2.5×10^8	1.7×10^8	3×10^8	1.2×10^8	2.8×10^8
200	1.1×10^8	2.6×10^8	1×10^8	3×10^8	1.1×10^8	2.8×10^8
400	8×10^7	1×10^8	1.7×10^8	9×10^7	4×10^7	7×10^7

The results (viable count of *Lactobacillus casei* L26) demonstrate that neither Teavigo™
nor *Strep. pyogenes* had a significant effect on the growth of *Lactobacillus casei* L26.

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C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional ☐	
We inform you that the availability of the microorganism identified above, referred to Rule 13bis PCT, shall be effected only by issue of a sample to an expert nominated by the requester until the publication of the mention of grant of the national patent or, where applicable, for twenty years from the date of filing if the application has been refused, withdrawn or deemed to be withdrawn.	
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B. IDENTIFICATION OF DEPOSIT	Further deposits are identified on an additional sheet ☐
Name of depositary institution CENTRAAL BUREAU VOOR SCHIMMELCULTURES	
Address of depositary institution (including postal code and country) Uppsalaalan 8 P.O. Box 85167 NL-3508 AD Utrecht The Netherlands	
Date of deposit 14 September 2005	Accession Number CBS 118529
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet ☐ We inform you that the availability of the microorganism identified above, referred to Rule 13bis PCT, shall be effected only by issue of a sample to an expert nominated by the requester until the publication of the mention of grant of the national patent or, where applicable, for twenty years from the date of filing if the application has been refused, withdrawn or deemed to be withdrawn.	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	

For receiving Office use only ☐ This sheet was received with the international application Authorized officer	For International Bureau use only ☐ This sheet was received by the International Bureau on: Authorized officer
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CLAIMS

1. Composition comprising a probiotic and a polyphenol.
- 5 2. Composition according to claim 1, wherein the polyphenol is a green tea extract.
3. Composition according to claim 2, wherein the green tea extract comprises at least 90% w/w, preferably at least 93% w/w, more preferably at least 95% w/w epigallocatechin gallate.
- 10 4. Composition according to any one of claims 1 to 4, wherein the probiotic is selected from the group consisting of a *Lactobacillus*, a *Bifidobacterium* species and mixture thereof.
- 15 5. Composition according to claim 4, wherein the *Lactobacillus* is selected from the group consisting of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus helveticus*, *Lactobacillus fermentum* and mixtures thereof and/or wherein the *Bifidobacterium* is selected from the group consisting of *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium lactis* and mixture thereof.
- 20 6. Composition according to claim 5, wherein the probiotica is selected from the group consisting of the *Lactobacillus acidophilus* CBS 116411, the *Lactobacillus casei*, CBS 116412, *Bifidobacterium lactis* CBS 118529.
- 25 7. Composition according to any one of claims 1 to 6, which has the capacity to kill an undesirable bacterium or to inhibit its growth, or to prevent the germination of its spores *in vitro* in a given medium and/or *in vivo* in a given food product matrix, crop protection application, pharmaceutical or cosmetic product.
- 30 8. Composition according to claim 7, wherein the undesirable bacterium is a Gram positive bacteria, preferably selected from the group consisting of a *Helicobacter*, *Streptococcus*, *Staphylococcus* species and mixture thereof.

9. Composition according to any one of claims 1 to 8, which additionally comprises a prebiotic.
- 5 10. Composition according to claim 9, wherein the prebiotic is selected from the group consisting of pentosans, b-glucans, pectins, and pectic polysaccharides, mannans, arabinans, galactans, fructo-oligosaccharides, resistant starch and mixture thereof.
- 10 11. Composition according to any one of claims 1 to 10 for use as a medicament.
12. Composition according to any one of claims 1 to 11 for preventing and/or treating infections caused by undesirable bacteria and associated diseases in a human being in a need thereof.
- 15 13. Composition according to claim 12, wherein the human being in a need of the medicament experiences some form of fatigue, stress, bed sores, gingivitis, nose and throat infection and peptic ulcer and mixture thereof.
- 20 14. Composition according to any one of claims 1 to 13, which is a preservative.
15. Composition according to claim 14, wherein the composition additionally comprises an anti-fungal agent selected from the group consisting of a weak acid preservative, a polyene anti-fungal compound, sulphur dioxide, sulphites, nitrate, nitrite, dimethyl dicarbonate, biphenyl, diphenyl, orthophenylphenol, thiobendazole, an inorganic acid, an imidazole and mixture thereof.
- 25 16. Product comprising the composition of any one of claims 1 to 15, wherein the product is selected from the group consisting of a medicament, a feed product, a food product, a cosmetic, an oral hygiene product and an agricultural product.
- 30 17. Use of a composition comprising a probioticum and a polyphenol as defined in any one of claims 1 to 16 for killing an undesirable bacterium or for inhibiting its

growth, or for preventing the germination of its spores *in vitro* in a given medium and/or *in vivo* in a given food product matrix, crop protection application, pharmaceutical or cosmetic product.

- 5 18. Use of a composition as defined in any one of claims 1 to 15 for the preparation of the product according to claim 16.
19. Use according to claim 18, for the preparation of a food product or a medicament for preventing and/or treating infections caused by undesirable bacteria and
10 associated diseases in a human being in a need thereof.
20. Use according to any one of claim 17 to 19, wherein the undesirable bacterium is a Gram bacterium, which is preferably selected from the group consisting of a *Helicobacter*, *Streptococcus*, *Staphylococcus* species and mixture thereof.
15
21. Use according to any one of claim 17 to 20, wherein the human being in a need of said food product of medicament is selected from the group consisting of: human subjects that experience some form of fatigue, stress, bed sores, gingivitis, nose and throat infection and peptic ulcer and mixture thereof.
20
22. Use according to claim 17, as a preservative for the preparation of the product according to claim 16.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP2005/055787

A. CLASSIFICATION OF SUBJECT MATTER

A61P31/04 A61K35/74 A61K36/82 A61K31/353

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, BIOSIS, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 018, no. 495 (C-1250), 16 September 1994 (1994-09-16) & JP 06 166626 A (TAIYO KAGAKU CO LTD), 14 June 1994 (1994-06-14)	1-16
Y	abstract	17-22
X	US 6 565 847 B1 (GORSEK WAYNE F) 20 May 2003 (2003-05-20)	1-16
Y	claims	17-22
X	GB 1 197 257 A (SEIJI KUWABARA) 1 July 1970 (1970-07-01)	1-16
Y	page 2, column 1, line 40 - line 50 page 2, column 2, lines 72-76; claims; example 1	17-22
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in 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

13 January 2006

Date of mailing of the international search report

31/01/2006

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Langer, A

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP2005/055787

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6 241 983 B1 (PAUL STEPHEN M ET AL) 5 June 2001 (2001-06-05) cited in the application column 1, line 59 - column 2, line 46 column 13, line 12 - line 38 -----	1-22
Y	YU C C ET AL: "In vitro inhibition of Helicobacter pylori by Bifidobacterium longum, acids and local drinks" ABSTRACTS OF THE GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 99, 1999, page 383, XP009059682 & 99TH GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY; CHICAGO, ILLINOIS, USA; MAY 30-JUNE 3, 1999 ISSN: 1060-2011 the whole document -----	1-22
Y	KABIR A M A ET AL: "PREVENTION OF HELICOBACTER PYLORI INFECTION BY LACTOBACILLI IN A GNOTOBIOTIC MURINE MODEL" GUT, BRITISH MEDICAL ASSOCIATION, LONDON,, GB, vol. 41, 1997, pages 49-55, XP000922761 ISSN: 0017-5749 cited in the application abstract -----	1-22
P,Y	LEE KEE-MYUNG ET AL: "Protective mechanism of epigallocatechin-3-gallate against Helicobacter pylori-induced gastric epithelial cytotoxicity via the blockage of TLR-4 signaling" HELICOBACTER, vol. 9, no. 6, December 2004 (2004-12), pages 632-642, XP002362244 ISSN: 1083-4389 cited in the application abstract -----	1-22
Y	CHAE SUCK-HEE ET AL: "Growth-inhibiting effects of Coptis japonica root-derived isoquinoline alkaloids on human intestinal bacteria" JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY, vol. 47, no. 3, March 1999 (1999-03), pages 934-938, XP002362245 ISSN: 0021-8561 abstract; table 2 -----	1-22
Y	US 5 071 653 A (KAKUDA ET AL) 10 December 1991 (1991-12-10) claims -----	1-22
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP2005/055787

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHUN J W ET AL: "Killing effects and inhibition of biofilm formation on human teeth against oral microorganisms by "green tea gargle" for dental care." ABSTRACTS OF THE GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 103, 2003, pages A-171 URL, XP009059683 & 103RD AMERICAN SOCIETY FOR MICROBIOLOGY GENERAL MEETING; WASHINGTON, DC, USA; MAY 18-22, 2003 ISSN: 1060-2011 the whole document	1-22
P,Y	CAO B H ET AL: "Effects of green tea polyphenols and fructo-oligosaccharides in semi-purified diets on broilers' performance and caecal microflora and their metabolites" ASIAN-AUSTRALASIAN JOURNAL OF ANIMAL SCIENCES, vol. 18, no. 1, January 2005 (2005-01), pages 85-89, XP001207867 ISSN: 1011-2367 Abstract page 87, paragraph 1; table 3	1-22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2005/055787

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 17 is directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

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

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP2005/055787

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
JP 06166626	A	14-06-1994	JP 3459837 B2	27-10-2003
US 6565847	B1	20-05-2003	NONE	
GB 1197257	A	01-07-1970	NONE	
US 6241983	B1	05-06-2001	NONE	
US 5071653	A	10-12-1991	JP 2211863 A	23-08-1990
			JP 2815628 B2	27-10-1998