

INDIRECT SUBSTRATES FOR MICROORGANISMS METABOLIZING 1,2-PROPANEDIOL

FIELD OF THE INVENTION

The present invention relates generally to enhancing the activity of certain probiotics in mammals. Moreover this invention relates to preparations comprising substrate components and certain probiotics, the substrate components being specifically designed to enhance the efficacy of said probiotics. The substrate components are selected to generate 1,2-propanediol, which uniquely most *Lactobacillus reuteri* strains can utilize as a source of energy and/or as an external electron acceptor.

BACKGROUND OF THE INVENTION

The Food and Agricultural Organization of the United Nations define probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”. Nowadays, a number of different bacteria are used as probiotics for example, lactic acid bacteria such as strains of *Lactobacillus* and *Bifidobacterium*.

The effectiveness of probiotics is strain-specific, and each strain may contribute to host health through different mechanisms. Different probiotics can prevent or inhibit the proliferation of pathogens, suppress production of virulence factors by pathogens, modulate the immune response in a pro-inflammatory or an anti-inflammatory way and influence the host in a number of other ways.

Prebiotics are defined as “non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one, or a limited number of bacteria in the colon that can improve the host health.” Targets for prebiotics are usually bifidobacteria and lactobacilli; however, prebiotics are often not selective, and hence stimulation of beneficial genera or probiotic strains alone may be difficult to achieve. Since it is difficult to find a prebiotic that is selective for certain probiotics, the inventor of the present invention has discovered how to use specific substrate components (SSC) that indirectly will supply specific probiotics with a source of energy and/or an external electron acceptor that will increase the energy yield.

Lactobacillus reuteri is a heterofermentative lactic acid bacterium and is frequently found in the gastrointestinal tract of humans and other animals. *L. reuteri* is considered an

indigenous organism of the human gastrointestinal tract and is for example present on the mucosa of the gastric corpus, gastric antrum, duodenum, and ileum. See, for example U.S. Patent Nos. 5,439,678, 5,458,875, 5,534,253, 5,837,238, and 5,849,289. When *L. reuteri* cells are grown under anaerobic conditions in the presence of glycerol, they produce the antimicrobial substance known as reuterin (β -hydroxy propionaldehyde). The ability to produce reuterin is due to the propanediol utilization (pdu) operon. The pdu operon is a metabolic machinery that also enables growth on 1,2-propanediol (PD). This pdu operon is a very important feature for *L. reuteri* when colonizing humans and exploiting the full potential of the bacteria. This machinery is rare among other lactobacilli and therefore those without the pdu-machinery are not able to grow on 1,2-PD and neither are they capable of using 1,2-PD as an electron acceptor.

Different *L. reuteri* strains have the ability to colonize the intestine, act as a diarrhea therapeutic agent, modulate the gut motility, function as an inhibitor of bacterial pathogens, immunologically modulate the gastrointestinal mucosa, function as an anti-inflammatory agent in the stomach etc.

In patent application WO2009/151391, the pdu machinery of *L. reuteri* is primed with 1,2-PD or glycerol before freeze-drying the bacteria. With this manufacture design the freeze-dried pdu machinery of *L. reuteri* is primed with the capacity to make and store reuterin.

Emma Årsköld et. al., *Phosphoketolase Pathway Dominates in Lactobacillus reuteri ATCC 55730 Containing Dual Pathways for Glycolysis* (Journal of Bacteriology, Jan 2008, p. 206-212) describes that growth performance of *L. reuteri* on glucose can be improved by adding fructose as an external electron acceptor. However nothing in this article teaches how to select certain SSCs based on their ability to indirectly supply certain probiotics with 1,2-PD, which may only be utilized by bacteria with the pdu-machinery.

A common problem with oral administration of probiotic bacteria is insufficient amounts and/or activity of the probiotic bacteria in locations of the intestinal tract where they will assert their effects. This may have as a consequence that the dosage of probiotic bacteria has to be increased and/or more frequent administration is needed and might also result in loss of activity. This leads to unnecessary costs, undesirable frequency of intake and/or decreased health benefits. In the present invention the local amounts and/or metabolic activity of for example *L. reuteri* is enhanced, leading for example to the possibility of lowering the dosage of the probiotic and further that site-directed health benefits are possible.

1,2-propanediol (1,2-PD) is a source of energy that can be locally produced by other coexisting microorganisms and utilized, possibly in combination with additional sugars, by

certain probiotic species, e.g. *L. reuteri*. The inventor of the present invention has surprisingly discovered that those coexisting microbes can be stimulated to produce 1,2-PD by oral administration of very selective SSCs and thereby indirectly enhance the activity of 1,2-PD utilizing organisms such as *L. reuteri*.

5 Pectin is a polysaccharide from plant cell walls. Various pectic polysaccharides can be detected in the cell wall, including homogalacturonan (HG), xylogalacturonan (XGA), apiogalacturonan, rhamnogalacturonan I (RGI), and rhamnogalacturonan II (RGII). The ratio between HG, XGA, RGI and RGII is variable, but typically HG is the most abundant polysaccharide constituting about 65% of the pectin, while RGI constitutes 20% to 35%.

10 XGA and RGII are minor components, each constituting less than 10%. The different pectic polysaccharides are not separate molecules but covalently linked domains. L-rhamnose is found as a constituent in the pectin structures RGI and RGII. L-fucose is also found as a constituent in the RGII structure. Bacteria found in the GI-tract that are able to convert L-rhamnose or L-fucose belong for example to Bacteroides and Enterobacteria genera, including
15 *E. coli* bacteria.

Pectin is resistant to human digestion, but is degraded to sugars and then further metabolized, for example to 1,2-propanediol, by bacteria in the small intestine and colon. Pectin stimulates bacterial growth in the small intestine and in the colon. Pectin is used as a remedy for diarrhea, is related to improved intestinal environment and is also known to have
20 anti-cancer properties. Modified citrus pectin (MCP) is citrus pectin that has been degraded to less complex molecules and is used to support cell growth and proliferation.

Fucoidan is a sulfated polysaccharide found mainly in various species of brown algae and brown seaweed such as mozuku, kombu, limu, moui, bladderwrack, wakame and hijiki, variant forms of fucoidan have also been found in animal species, including the sea cucumber.

25 Galacto-oligosaccharides (GOS) generally comprise a chain of galactose units that arise through consecutive transgalactosylation reactions, with a terminal glucose unit, is classified as a prebiotic.

Lynch MB et al., *The effect of dietary Laminaria-derived laminarin and fucoidan on nutrient digestability, nitrogen utilisation, intestinal microflora and volatile fatty acid*
30 *concentration in pigs* (J Sci Food Agric. 2010 Feb;90(3):430-7) have seen that pigs offered diets containing fucoidan have increased *Lactobacillus spp.* in the proximal colon and distal colon compared with non-fucoidan diets. Thus it is suggested that fucoidan may provide a dietary means to improve gut health in pigs. The increased *Lactobacillus* populations in feces due to a fucoidan diet have also been seen by J.V. O'Doherty et. al., *The effect of dietary*

laminarin and fucoidan diet of the weanling piglet on performance and selected faecal microbial populations (Livestock science 2010 September).

However it was not previously known to select, for example, pectin and fucoidan, or fractions thereof, based on the amounts of L-rhamnose and/or L-fucose in order to generate 1,2-PD through bacterial fermentation and to use such compositions with high deoxy sugar content, particularly high L-rhamnose and/or L-fucose content which will lead to high amounts of 1,2-PD thus indirectly supplying certain microorganism, for example *L. reuteri* with a source of energy and/or an external electron acceptor, which most other microbes are not able to utilize due to lack of the pdu machinery.

Patent application WO2010/117274 relates to a carbohydrate which is able to induce a detectable increase of a C5 and/or a C6 Short Chain Fatty Acid (SCFA). The SCFA has a positive effect on the gastrointestinal health of the subject treated. The carbohydrate used comprises pectin. Even though they chose pectins that may comprise traces of rhamnose, they do not disclose how to select and use specific pectin high in L-rhamnose or L-fucose to indirectly supply probiotics with the pdu-machinery with a specific source of energy and/or an external electron acceptor thus enhancing their activity.

U.S. Patent No. 7,101,565 relates to a composition comprising a prebiotic and a probiotic. The prebiotic may comprise a pectin or pectic polysaccharide. However it is not disclosed in this invention how to select certain pectins, or use combinations with pectin and L-rhamnose or L-fucose, that will generate high amounts of 1,2-PD in the gastrointestinal tract, beneficial for probiotics with the pdu machinery.

In U.S. Patent No. 5,902,578 an invention is disclosed that relates to a method of preventing diarrhea associated with infectious agents such as rotavirus, or diarrhea associated with antibiotic therapies by using *Lactobacillus*. However in this invention *Lactobacillus* is not associated with additional SSCs for better efficacy.

Nobody has hitherto disclosed how to enhance the health promoting effects of certain probiotics by administering SSCs, together with a probiotic, e.g. *L. reuteri*, to indirectly supply such probiotics with a unique source of energy and/or an external electron acceptor. Oral administration of SSCs, with high content of L-rhamnose and/or L-fucose will secure the supply of 1,2-propanediol and thus indirectly supply certain prebiotics with a source of energy and/or an external electron acceptor. This will increase the local amounts of health promoting microorganisms, e.g. *L. reuteri*, and provide better efficacy, making site directed effects possible.

Even though it has previously been known to use for example pectin together with

probiotics, it is not previously known how to select SSCs based on their ability to form 1,2-PD for the indirect supply of certain probiotics with a specific energy source and/or a specific external electron acceptor.

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SUMMARY OF THE INVENTION

This invention discloses a method of enhancing the activity of certain probiotics and the manufacturing and use of products, which comprises substrate components and optionally a probiotic. The products of the present invention can be used to enhance the activity of for example *L. reuteri* in mammals. This product can be used for improving the host health.

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Depending on the used probiotic strain, the product can be used for example to improve gastrointestinal health, improve immune-related health, treat and/or prevent diarrhea and constipation, normalize fecal consistency, improve gastrointestinal motility, treat and/or prevent infectious diseases, modulate inflammation and anti-pathogenic effect.

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The increased efficacy of probiotics can be achieved by stimulating co-existing microbes to produce 1,2-propanediol (1,2-PD). The coexisting microbes are stimulated with certain specific substrate components (SSC) as described herein. The SSCs will ensure the presence of 1,2-PD in the gastro intestinal tract and indirectly supply certain beneficial organisms with 1,2-PD.

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The ability to utilize 1,2-PD either as an energy source and/or as an external electron acceptor is unique for bacteria with the pdu-machinery and therefore the administration of SSCs will enhance the activity only of certain probiotics.

The SSCs can be administered together with the probiotics for enhancing the activity of the co-administered probiotics. The SSCs could also be administered alone, for example to increase the activity of previously administered probiotics.

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1,2-PD, administered alone or generated by the SSCs, may further be combined with galacto-oligosaccharides (GOS) or other galactose containing saccharides to give an even better source of energy for the microorganisms.

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Heterofermentative lactic acid bacteria produce lactate, ethanol and carbon dioxide using the phosphoketolase pathway (PKP). The PKP has poor energy yield compared to the Embden-Meyerhof pathway (EMP). This disadvantage can be compensated for by addition of external electron acceptors.

The inventor has surprisingly found out that by ensuring the presence of 1,2-propanediol in the gastrointestinal tract, the probiotics will simultaneously be supplied with a suitable external electron acceptor and thereby enhance the activity of the probiotic.

The SSCs of the present invention will selectively increase the growth of heterofermentative lactic acid bacteria, such as *Lactobacillus reuteri*, since they will provide the bacteria with a suitable electron acceptor enabling an enhanced activity.

L. reuteri are dependent on a good electron acceptor for growth in certain environments, and the inventor of the present invention has surprisingly found out that 1,2-propanediol will serve as a good electron acceptor and can be supplied by the administration of SSCs.

According to one aspect of the invention, a method is provided for enhancing the activity of probiotic bacteria having a pdu-operon, in the gastrointestinal tract of an individual, comprising administering a substance to said individual, which substance has the capacity to be metabolized to 1,2-propanediol in the gastrointestinal tract of said individual.

In an embodiment of the method, the substance comprises a deoxy sugar, which has the capacity to be metabolized to 1,2-propanediol in the gastrointestinal tract of said individual. In an embodiment, the deoxy sugar is rhamnose or fucose.

In an embodiment of the method, the substance comprises (a) rhamnose, (b) fucose, (c) pectin having a high percentage of rhamnose, (d) rhamnose in combination with pectin, (e) fucose in combination with pectin, (f) fucoidan having a high percentage of fucose, or (g) a combination of rhamnose, fucose and pectin.

In an embodiment of the invention, pectin having a high percentage of rhamnose is defined as comprising 5-15% rhamnose, such as 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 % rhamnose. In an embodiment of the invention, fucoidan having a high percentage of fucose is defined as comprising more than 15 % fucose.

In an embodiment of the method, the substance is administered simultaneously with bacteria having a pdu operon.

In a preferred embodiment of the invention, the substance is administered orally to the individual.

In another preferred embodiment of the method, the bacteria having the pdu-operon comprise *Lactobacillus reuteri*.

In an embodiment of the method, the substance is administered to the individual at a daily dose of 0.25-25 g, preferably 1-2 g.

In an embodiment of the invention, the method further comprises simultaneously administering a galactooligosaccharide or other saccharides comprising galactose.

According to a second aspect of the invention, a substance is provided for use in enhancing the activity or increasing the growth of probiotic bacteria having a pdu-operon, in

the gastrointestinal tract of an individual, which substance comprises a deoxy sugar that has the capacity to be metabolized to 1,2-propanediol in the gastrointestinal tract of said individual.

In an embodiment, the deoxy sugar is rhamnose or fucose.

5 In an embodiment of the invention, the substance comprises (a) rhamnose, (b) fucose, (c) pectin having a high percentage of rhamnose, (d) rhamnose in combination with pectin, (e) fucose in combination with pectin, (f) fucoidan having a high percentage of fucose, or (g) a combination of rhamnose, fucose and pectin.

10 In an embodiment of the invention, pectin having a high percentage of rhamnose is defined as comprising 5-15% rhamnose, such as 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 % rhamnose. In an embodiment of the invention, fucoidan having a high percentage of fucose is defined as comprising more than 15 % fucose.

In a preferred embodiment relating to the substance, the bacteria having the pdu-operon are *Lactobacillus reuteri*.

15 In an embodiment of the invention, the substance is administered to the individual at a daily dose of 0.25-25 g, preferably 1-2 g.

In an embodiment of the invention, the substance is for use in combination with galactooligosaccharides or other saccharides comprising galactose.

20 According to a third aspect of the invention, a composition is provided comprising (i) bacteria having a pdu-operon and (ii) a substance comprising a deoxy sugar, which deoxy sugar has the capacity to be metabolized to 1,2-propanediol in the gastrointestinal tract of an individual.

25 In an embodiment, the composition comprises bacteria having a pdu-operon, in combination with (a) rhamnose, (b) fucose, (c) pectin having a high percentage of rhamnose, (d) rhamnose in combination with pectin, (e) fucose in combination with pectin, (f) fucoidan having a high percentage of fucose, or (g) a combination of rhamnose, fucose and pectin.

30 In an embodiment of the invention, pectin having a high percentage of rhamnose is defined as comprising 5-15% rhamnose, such as 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 % rhamnose. In an embodiment of the invention, fucoidan having a high percentage of fucose is defined as comprising more than 15 % fucose.

In another embodiment, the composition further comprises galactooligosaccharides or other saccharides comprising galactose.

In an embodiment relating to the composition, the bacteria having the pdu-operon are *Lactobacillus reuteri*.

In an embodiment of the composition, the substance is present in an amount such as to give a daily dose of 0.25-25 g, preferably 1-2 g.

According to another embodiment of the invention, the above-described composition is for use in enhancing the activity or increasing the growth of probiotic bacteria having a pdu-operon, in the gastrointestinal tract of an individual.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 is a graph showing growth of *L. reuteri* DSM 17938 in modified MRS (with no glucose and citrate) with addition of 1,2-PD, galactose and a combination thereof.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS THEREOF

The propanediol utilization (pdu) machinery of certain probiotics, among them for example *Lactobacillus reuteri*, enables growth on 1,2-propanediol (1,2-PD) as energy source and enables the ability to use 1,2-PD as an external electron acceptor.

The inventor of the present invention has surprisingly found a way of enhancing the activity of certain probiotics, using certain specific substrate components (SSCs) to indirectly support a specific probiotic organism with 1,2-PD that may be utilized as a source of energy or as an external electron acceptor.

The SSC is a substance. According to one embodiment of the invention, the substance is a substrate. In one embodiment, the substance consists of one component only. In another embodiment, the substance comprises two or more components.

Oral administration of these carefully selected SSCs stimulates coexisting microbes to produce 1,2-PD, which leads to locally produced 1,2-PD that can serve as a source of energy or as an external electron acceptor for certain microorganisms with the pdu-machinery, such as for example *L. reuteri*. The SSCs may be administered alone or together with the probiotics.

The presence of 1,2-PD will improve the growth conditions for certain microorganisms, moreover the inventor has found out that this can be further enhanced in the presence of galacto-oligosaccharides (GOS) or galactose, as can be seen in figure 1.

The SSCs of the present invention are chosen for their ability to indirectly support a specific probiotic with 1,2-PD, which may be used as energy source or as an external electron

acceptor. The inventor of this invention has discovered that SSCs with high amounts of L-rhamnose or L-fucose are the most effective when used to enhance the activity of certain probiotics. Therefore the SSCs used in this invention are carefully selected with regards to the amounts of L-rhamnose and L-fucose.

5 Pectins, preferably certain fractions of pectin comprising high percentages of L-rhamnose, such as rhamnogalacturonan I and II, may be used as SSCs. Preferably, these preferred fractions of pectin comprise 5-15% rhamnose. These fractions of pectin will, when degraded, result in more rhamnose than unfractionated pectin, which in the present text may be called pectin, ordinary pectin, or regular pectin. A certain daily dose of such preferred
10 fractions of pectin, e.g. 2 g, will thus generate higher amounts of 1,2-PD as compared to the same daily dose (2 g) of regular pectin. Ordinary pectin could also be used, if administered together with L-rhamnose or L-fucose to indirectly and in the same manner be advantageous for certain microbes, e.g. *L. reuteri*. This combination will, in addition to 1,2-PD, also supply certain probiotics with other substrates, e.g. galactose, arabinose and galacturonic acid as a
15 result of pectin degradation. The inventor of the present invention has shown that 1,2-PD in combination with certain pectin constituents, e.g. galactose, arabinose and galacturonic acid will generate a synergistic effect that enhances the utilization of 1,2-PD for certain probiotics, e.g. *L. reuteri*, as seen in figure 1.

 Fucoidan, preferably certain fractions of fucoidan comprising high amounts of L-
20 fucose may be used as SSCs in the present invention, and preferably these fractions of fucoidan comprise more than 15 % fucose. These fractions of fucoidan will, when degraded, result in more fucose than ordinary fucoidan and thus generate higher amounts of 1,2-PD.

 L-rhamnose or L-fucose alone may also be used alone as SSCs of this invention.

 Other SSCs, e.g. gums and other polysaccharides, can be used according to the present
25 invention if they contain L-rhamnose, L-fucose or the like. Gums include, but are not limited to karaya gum and arabic gum. Further, some human milk oligosaccharides (HMO's) from human breast milk can be used as SSCs in the present invention.

 Administering the SSCs of the present invention, either alone or together with a probiotic, e.g. *L. reuteri*, secures the supply of energy source for a specific probiotic and/or
30 secures the presence of an external electron acceptor that will increase the energy yield, thus enhancing the local activity and efficacy of said probiotic.

 In other embodiments and to support this effect it is further possible to add GOS or galactose in order to increase *L. reuteri*'s ability to utilize 1,2-PD.

 Depending on the target indication, a number of *L. reuteri* strains can be used in the

invention herein with different ability to colonize the intestine, act as a diarrhea therapeutic agent, modulate the gut motility, function as an inhibitor of bacterial pathogens, immunologically modulate the gastrointestinal mucosa, function as an anti-inflammatory agent in the stomach etc.

5 Pectin, L-rhamnose and L-fucose are resistant to human digestion, but are degraded to sugars and then further metabolized to for example 1,2-propanediol by co-existing microbes found in humans and available at certain locations of the human GI-tract, for example on the mucosa of the gastric corpus, gastric antrum, duodenum, and small intestine. The invention herein therefore also makes it possible to favor site-directed effects in the human GI-tract. For
10 example by using selected strains of *L. reuteri* as the probiotic, it is possible to enhance the anti-inflammatory effect of this strain in the ileum.

When using the described system of certain SSC's in the present invention, with for example a specific strain of *L. reuteri* in humans with a very disturbed microflora, including a lack of normally found co-existing microbes able to convert the SSC to 1,2-PD, it is also
15 another possibility of the invention herein to actively supply such co-existing microorganisms, e.g. *Lactobacillus rhamnosus*, to the recipient ensuring the efficacy of the administered SSCs.

The products comprising the SSCs of this invention, alone or in combination with certain probiotics are preferably formulated as a tablet, capsule, powder sachet or the like.
20 The product can be a food-supplement, a pharmaceutical product or the like. In such a product, the amount of probiotic feed should be in an amount sufficient to give the wanted effect of the specific strain, now also considering the enhanced effect by the SSC. Such levels are typically, but not limited to 10E+4 CFU to 10E+11 CFU per day, preferably in the range of 10E+6 CFU to 10E+9 CFU of *L. reuteri*.

25 The amount of the SSC should be in the range of 0.25 to 25 grams (g) per day when using pectins with high amounts of L-rhamnose and/or L-fucose. When using a combination of regular pectin with separate L-rhamnose and/or L-fucose, the total amount of SSCs should be in the range of 0.25 to 25g per day. In both cases, the daily dose of 0.25-25 g may for example be 0.25, 0.5, 1, 1.5, 2, 5, 10, 15, 20 or 25 g, preferably 1-2 g, such as 1, 1.25, 1.5,
30 1.75 or 2 g.. The ratio between the regular pectin and either one of L-rhamnose or L-fucose, or the combination thereof, should be in the range of 95:5 to 0:100, but preferably 80:20 to 20:80 and even more preferably 70:30.

Another option when using the invention herein is to alternatively feed the SSC and the probiotic together, and at one or more occasions following the first feeding to feed only

the SSC in a kind of shuttle program to lower the cost of treatment.

It is essential for this invention that the probiotics used have the pdu-machinery, since this is essential for the ability to use 1,2-PD as a source of energy and/or as an external electron acceptor. Therefore in another embodiment of the invention the pdu machinery of the probiotics is primed with 1,2-PD during the production of the probiotic strain for enhanced efficacy. This is done by adding 1,2-PD or glycerol and possibly cobalt or vitamin B-12 (since vitamin B-12 and cobalt are important for reuterin production) at the start of the fermentation step when culturing the bacteria. With this manufacture design, the freeze-dried bacteria to be used in the next step are better prepared to more quickly activate the pdu-machinery. This enhanced efficacy of the pdu machinery will in turn enhance the efficacy of the administered SSC of the present invention. Another way of enhancing the efficacy of the administered SSCs is to combine them with GOS or galactose, and the inventor has shown that the combination of 1,2-PD and galactose has an unexpected benefit on *L. reuteri*'s growth.

Since numerous modifications and changes will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and operation shown and described, and accordingly, all suitable modifications and equivalents may be resorted to, falling within the scope of the invention as defined by the appended claims.

EXAMPLE 1

Manufacture of a sachet containing a composition of L. reuteri together with pectin, rhamnose and galacto-oligosaccharides.

The composition is made of:

L. reuteri DSM 17938: 10E+8 CFU / sachet

Pectin (GENU® pectin (citrus) type USP/200, CP Kelco France SARL, France): 840 mg / sachet

L-rhamnose : (Rhamnose monohydrate L-(+), Kaden Biochemicals GmbH, Hamburg, Germany) 360 mg / sachet

GOS15 (VIVINAL®, FrieslandCampina Domo, The Netherlands) 800 mg / sachet

The composition is filled at ambient temperature into aluminum foil bags as known in the art with desiccant (10 cm x 12 cm, using packaging material PET12/PE/ALU 12/PE/PE+desiccant/PE from Alcan) in a LAF bench (Holten Laminair Model S-2010 1.2 from Heto-Holten A/S, Denmark). To each bag, 2 g of powder with *L. reuteri*, pectin, L-rhamnose and galacto-oligosaccharides is added using the balance XP-600 from Denver

Instrument GmbH, Germany. The filled aluminum foil bags are then heat sealed with the film sealing device model F460/2 from Kettenbaum Folienschweisstechnik GmbH & Co. KG, Germany.

5 **EXAMPLE 2**

Manufacture of a sachet containing a composition of L. reuteri together with rhamnose.

The composition is made of:

2 g L-rhamnose: (Rhamnose monohydrate L-(+), Kaden Biochemicals GmbH, Hamburg, Germany)) containing 10E+8 CFU *L. reuteri* DSM 17938 / sachet

10 The composition is filled into aluminum foil bags as in Example 1.

EXAMPLE 3

Manufacture of a sachet containing a composition of L. reuteri together with galacto-oligosaccharides and rhamnose.

15 The composition is made of:

1 g GOS15 (VIVINAL®, FrieslandCampina Domo, The Netherlands) and 1 g L-rhamnose: (Rhamnose monohydrate L-(+), Kaden Biochemicals GmbH, Hamburg, Germany) containing 10E+8 CFU *L. reuteri* DSM 17938 / sachet

The composition is filled into aluminum foil bags as in Example 1.

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EXAMPLE 4

Manufacture of a primed L. reuteri strain

This example describes how to manufacture a freeze-dried powder of *L. reuteri* with activated pdu-machinery. The primed *L. reuteri* strain can then be used when producing the sachets of examples 1-3.

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Fermentation medium composition

Dextrose mono hydrate 60 g/l

Yeast extract KAV 20 g/l

Peptone Type PS (of pig origin) 20 g/l

30 Di ammonium hydrogen citrate 5 g/l

Sodium acetate (x 3 H₂O) 4.7 g/l

Di potassium hydrogen phosphate 2 g/l

Tween80 0.5 g/l

Silibione (anti foam) 0.14 g/l

Magnesium sulphate 0.10 g/l
Manganese sulphate 0.03 g/l
Zinc sulphate hepta hydrate 0.01 g/l
Water q.s.

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Centrifuge medium

Peptone O-24 Orthana (of pig origin)

Cryoprotectants

Lactose (of bovine origin) 33 %
Gelatin hydrolysate (of bovine origin) 22 %
Sodium glutamate 22 %
Maltodextrin 11 %
Ascorbic acid 11 %

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Production steps of freeze dried Lactobacillus reuteri powder

1. Twenty ml of the fermentation medium is inoculated with 0.6 ml of freeze-dried *Lactobacillus reuteri* powder from a working cell bank vial. The fermentation is performed in a bottle at 37°C for 18 – 20 hours without stirring or pH control *i.e.* static.

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2. Two 1-liter flasks of the fermentation medium are inoculated with 9 ml cell slurry (from step 1) per liter of medium. The fermentation is performed at 37°C for 20 – 22 hours without stirring or pH control *i.e.* static.

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3. The two one liter cell slurries from step no. 2 are used to inoculate the 600-liter vessel containing 600 liters fermentation medium. The fermentation is performed at 37°C for 13 hours with stirring and pH control. At the start of the fermentation the pH is 6.5. The pH control starts when the pH drops below 5.4 using a 20% sodium hydroxide solution. The pH control is set to pH 5.5.

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4. The fourth and final fermentation step is performed in a 15,000-liter vessel with the inoculation from step no. 3. The fermentation is performed at 37°C for 9 to 12 hours with stirring and pH control. At the start of the fermentation the pH is 6.5. The pH control starts when the pH drops below 5.4 using a 20% sodium hydroxide solution. The pH control is set to pH 5.5. 100 mM glycerol is added in the final phase of the fermentation, just before the culture reaches the stationary phase. The fermentation is complete when the culture reaches the stationary phase, which can be seen by the reduction of the addition of the sodium

hydroxide solution. Roughly 930 liters of the sodium hydroxide solution is added to the 10,200 liters of media and 600 liters of inoculum during the fermentation.

The cell slurry from the final fermentation (step 4) is separated at 10°C twice in a continuous centrifuge from Alfa Laval. By the first centrifugation the volume of the cell slurry is reduced from roughly 11,730 liters to 1200 liters. This volume is washed with 1200 liters of a peptone (Peptone 0-24, Orthana) solution in a 3000-liter vessel and is separated again before the mixing with the cryoprotectants (see below). The washing step with peptone is performed to avoid any freezing-point reduction in the freeze-drying process.

By the second centrifugation the volume of the cell slurry is reduced to 495 liters. This volume is mixed with 156 kg of the cryoprotectant solution to reach roughly 650 liters of the cell slurry.

The cell slurry is pumped to a 1000-liter vessel. The vessel is then transported to the freeze-drying plant.

At the freeze-drying plant, exactly 2 liters of the cell slurry is poured on each plate in the freeze dryer. The maximum capacity of the freeze dryer is 600 liters and all excessive cell slurry volume is thrown away.

The cell slurry of *Lactobacillus reuteri* has a dry matter content of 18 % and is freeze dried for four to five days.

During the freeze-drying process, the pressure in the process is between 0.176 mbar and 0.42 mbar. The vacuum pump is started when the pressure reaches 0.42 mbar. The PRT (pressurizing test) is used to determine when the process is complete. If the PRT or the increase of pressure is less than 0.02 mbar after 120 seconds, the process is stopped.

EXAMPLE 5

Combination of 1,2-PD and galactose generates a synergistic effect enhancing the activity of L. reuteri

L. reuteri DSM 17938 was grown in modified MRS broth (with no glucose and citrate) with addition of 1,2-PD (0.3%), galactose (0.3%) or a combination of the two. The bacteria were grown for 24 h at 37°C. *L. reuteri* grown in the presence of both 1,2-PD and galactose surprisingly showed a significantly higher growth than the growth on the separate substances as seen in figure 1.

EXAMPLE 6

Example of shuttle program to be fed

Thanks to the enhanced activity of *L. reuteri* induced by the SSCs described in this application it is possible to alternate the sachets of example 1 (sachet A) with sachets where *L. reuteri* is excluded but otherwise manufactured according to example 1 (sachet B) in a shuttle program. This shuttle program does not reduce the efficiency of *L. reuteri* and may lower the treatment cost.

Sachet A is administered to the recipient at day 1, at day 2 and 3 the recipient is given sachet B. This administration scheme is repeated during the whole treatment period.

EXAMPLE 7

Intestinal colonization in vivo in humans

A comparison between the intestinal colonization by *L. reuteri* alone and the same *L. reuteri* administered together with SSC is made in a clinical study. 12 healthy volunteers are divided into two groups (A and B) with 6 participants in each group. Group A receives the powder sachets of example 1, containing a composition of *L. reuteri* together with SSCs in the form of pectin, rhamnose and galacto-oligosaccharides. Group B receives the same *L. reuteri* strain but none of the above SSCs. Both groups are given $10E+8$ CFU of *L. reuteri* per day during 60 days. The quantitative evaluation of intestinal colonization by strains given alone or together with the SSCs is made by fecal sample examination at the beginning of the study, and after 30 and 60 days of the treatment period. Fecal *L. reuteri* is counted and the fecal amounts of group A and B are compared.

A significant increase in the fecal amounts of *L. reuteri* is seen in patients where *L. reuteri* is administered together with SSCs, compared to patients where *L. reuteri* is administered alone, as seen in table 1. The values are given as the average $\log_{10}$ CFU per gram of feces $\pm$ SEM.

Table 1

study group (n)	<i>L. reuteri</i> count		
	before	day 30	day 60
group A (6)	N.D.	5.5 ± 0.2	5.8 ± 0.3
group B (6)	N.D.	4.2 ± 0.3	4.5 ± 0.4

CLAIMS

1. A method for enhancing the activity of probiotic bacteria having a pdu-operon, in the gastrointestinal tract of an individual, comprising administering a substance to said individual, which substance has the capacity to be metabolized to 1,2-propanediol in the gastrointestinal tract of said individual,

the substance comprising of a) rhamnose, (b) fucose, (c) pectin having a high percentage of rhamnose, (d) rhamnose in combination with pectin, (e) fucose in combination with pectin, (f) fucoidan having a high percentage of fucose, or (g) a combination of rhamnose, fucose and pectin; and further comprising galactooligosaccharides or other saccharides comprising galactose;

wherein the substance is administered to the individual at a daily dose of 1-2 g and at the same time administer to the individual $10E+6$ CFU to $10E+9$ CFU of *L. reuteri* bacteria.

2. The method of claim 1, wherein the substance is administered to the individual at a daily dose of 0.25-25 g, preferably 1-2 g.

3. A composition comprising *L reuteri* bacteria having a pdu-operon and a substance comprising a deoxy sugar, which deoxy sugar has the capacity to be metabolized to 1,2-propanediol in the gastrointestinal tract of an individual, said bacteria having a pdu-operon, in combination with (a) rhamnose, (b) fucose, (c) pectin having a high percentage of rhamnose, (d) rhamnose in combination with pectin, (e) fucose in combination with pectin, (f) fucoidan having a high percentage of fucose, or (g) a combination of rhamnose, fucose and pectin; and further comprising galactooligosaccharides or other saccharides comprising galactose;

wherein the substance is present in an amount such as to give a daily dose of 0.25-25 g, preferably 1-2 g.