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(54) Title: VITAMIN B2 FOR USE IN IMPROVING GUT HEALTH

(57) Abstract: The present invention relates to vitamin B2 (riboflavin) for use in improving gut health in animals and humans. In particular, it was found that vitamin B2, upon delivery to the large intestine, increases the abundance of the beneficial bacteria *Bacillus coagulans* in the intestinal tract.

VITAMIN B2 FOR USE IN IMPROVING GUT HEALTH

FIELD OF THE INVENTION

The present invention relates to vitamin B2 (riboflavin) for use in improving gut health in animals
5 and humans. It was found that vitamin B2, upon delivery to the large intestine, increases the abundance of the beneficial bacteria *Bacillus coagulans* in the intestinal tract.

BACKGROUND OF THE INVENTION

Increasing evidence indicates that imbalances in the human gut microbiota (also referred to as
10 “dysbiosis”) may be associated with Western diseases, including obesity and type 2 diabetes, as well as cardiovascular, autoimmune, and intestinal inflammatory disease. Thus, targeted modulation of the human gut microbiome intended to restore imbalances represents a potential therapeutic and preventive strategy and has attracted the attention of academics as well as those engaged in various industries. Public awareness and acceptance of substances that modulate
15 the human gut microbiome continue to grow.

There is a consensus that certain live microorganisms, so called probiotics, have beneficial effects on human health. *Bacillus coagulans* is a highly efficacious and safe, spore-forming probiotic which has benefits for digestive health (Kalman DS et al. “A Prospective, Randomized, Double-blind, Placebo-controlled Parallel-group Dual Site Trial to Evaluate the Effects of a *Bacillus coagulans*-based Product on Functional Intestinal Gas Symptoms” (2009); Hun L., *Bacillus coagulans* Significantly Improved Abdominal Pain and Bloating in Patients With IBS” (2009); Dolin BJ., Effects of A Proprietary *Bacillus coagulans* Preparation on Symptoms of Diarrhea-Predominant Irritable Bowel Syndrome (2009)), and immune support (Anaya-Loyola MA et al.,
20 *Bacillus coagulans* GBI-30, 6086 decreases upper respiratory and gastrointestinal tract symptoms in healthy Mexican scholar-aged children by modulating immune-related proteins (2019); Kimmel M et al., A Controlled Clinical Trial to Evaluate the Effect of BC30 on Immunological Markers (2010)).

30 Recently, it was demonstrated that vitamins may modulate the human gut microbiome. WO2020/043797 discloses that vitamins can be useful to increase the growth of certain beneficial bacteria in the intestine. However, the human gut is home to hundreds of different microbes, and it would be desirable to be able to boost specific beneficial bacteria. In particular, it would be

desirable to increase the abundance of *Bacillus coagulans* in the intestine to enhance wellness, improve digestive health, and support the immune system.

SUMMARY OF THE INVENTION

- 5 The present invention relates to the following items:
- 1) Vitamin B2 for use in increasing the abundance of *Bacillus coagulans* in the intestine of an animal, preferably a human, wherein said use comprises delivering the vitamin B2 to the large intestine.
 - 2) Vitamin B2 for the use according to item 1, wherein the vitamin B2 is delivered to the large
10 intestine by a delayed-release formulation.
 - 3) Vitamin B2 for the use according to item 1 or 2, wherein said use comprises administering to the animal a formulation comprising a vitamin B2 dose of up to 200 mg/day, preferably 5-100 mg/day, more preferably 10-50 mg/day.
 - 4) Vitamin B2 for the use according to any one of items 1-3, wherein *Bacillus coagulans* is co-
15 administered with vitamin B2.
 - 5) Vitamin B2 for the use according to any one of items 1-4, wherein the animal, including a human, is experiencing a condition selected from the group consisting of: irritable bowel syndrome, functional intestinal gas symptoms, diarrhea, abdominal pain and bloating, upper respiratory symptoms, airway infections.
 - 20 6) Vitamin B2 for the use according to any one of items 1-5, wherein the *Bacillus coagulans* is *Bacillus coagulans* BC30.
 - 7) An oral formulation comprising vitamin B2 and *Bacillus coagulans*.
 - 8) The oral formulation according to item 7 which is a delayed-release formulation
 - 9) A method of increasing the abundance of *Bacillus coagulans* in the intestine of an animal,
25 preferably a human, comprising administering to the animal a formulation comprising an effective dose of vitamin B2.
 - 10) The method according to item 9, wherein the animal is a human and the vitamin B2 is delivered to the large intestine.
 - 11) The method according to item 9 or 10, wherein the vitamin B2 is delivered by a delayed-
30 release formulation.
 - 12) The method according to any one of claims 9-11, wherein *Bacillus coagulans* is co-administered with vitamin B2.
 - 13) The method according to any one of items 9-12, which is a method of treating, preventing, or lessening the symptoms of irritable bowel syndrome, functional intestinal gas symptoms,

diarrhea, abdominal pain and bloating, upper respiratory symptoms, airway infections in an animal, including a human, in need thereof.

14) The method according to any one of items 9-13, wherein the *Bacillus coagulans* is *Bacillus coagulans* BC30.

5

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the *Bacillus coagulans* BC30 levels (ComK gene copies/mL) ($\pm$ stdev) following 48h of colonic incubation, averaged over six different human donors. Average results are shown for *Bacillus coagulans* BC30 alone or when co-supplemented with vitamin B2. Statistically significant differences between the test conditions and the control are indicated with '*' ($p < 0.05$).

10

DETAILED DESCRIPTION OF THE INVENTION

Bacillus coagulans is a bacterial strain known for its beneficial effects on human health, and gut health in particular. The present inventors have found that vitamin B2 can boost the growth of *Bacillus coagulans*, leading to an increase of *Bacillus coagulans* levels in the gut.

15

Hence, in a first aspect, the present invention relates to vitamin B2 for use in improving gut health in an animal. Said improvement comprises or consists of increasing the abundance of *Bacillus coagulans* in the intestine of said animal. Specifically, the vitamin B2 is for use in increasing the abundance of *Bacillus coagulans* in the large intestine (colon) of an animal, and preferably, said use comprises delivering the vitamin B2 to the large intestine. Preferably, the animal is a human.

20

Vitamin B2 (also known as riboflavin) is one of the water-soluble B vitamins which is an essential component of two major coenzymes, flavin mononucleotide (FMN; also known as riboflavin-5'-phosphate) and flavin adenine dinucleotide (FAD). These coenzymes play major roles in energy production; cellular function, growth, and development; and metabolism of fats, drugs, and steroids. Riboflavin can be purchased from DSM GmbH. Alternative suppliers are TER Chemicals Distribution Group, BIOCHEM Bernburg GmbH, DVA International GmbH, Falken Trade GmbH, and Neupert Ingredients GmbH.

25

30

The most common *Bacillus coagulans* strain is *Bacillus coagulans* BC30 (also known as *Bacillus coagulans* GBI-30, 6086; or "BC30"). BC30 can be purchased, for example, from KERRY (Kerry Group, Ireland), or from Ganeden Biotech, USA. GanedenBC30™ is contained in dietary

supplements which are available, for example, from Schiff (Digestive Advantage, Daily Probiotic Capsule).

Alternative *Bacillus coagulans* strains are, for example, *Bacillus coagulans* MTCC 5856 and
5 *Bacillus coagulans* 15B. *B. coagulans* MTCC 5856 has been in the market as a dietary ingredient for nearly two decades, under the trade name LactoSpore®. The active product *B. coagulans* MTCC 5856 is supplied, for example, by Sabinsa Corporation, Utah, USA. *Bacillus coagulans* 15B can be purchased, for example, from Green Jeeva, USA.

10 To achieve an increase of the abundance of *Bacillus coagulans* in the large intestine, the vitamin B2 is preferably directly delivered to the large intestine. That is, the vitamin is delivered/ administered in a manner such that the vitamin is not absorbed in the stomach and/or small intestine; rather the vitamin is delivered/ administered to the distal intestinal tract, preferably the large intestine (colon). This is preferably done by delivering/ administering the vitamin B2 in a
15 delayed-release formulation. Oral administration is preferred.

Preferably, the vitamin B2 dose administered to the animal is up to 200 mg/day, preferably 5-100 mg/day, more preferably 10-50 mg/day. In one embodiment, vitamin B2 is administered in an amount such that its local concentration in the colon is at least 0.001 g/L, preferably at least 0.01
20 g/L more preferably at 0.02 g/L. Preferred local concentrations in the colon range from about 0.001 g/L to about 0.5 g/L, or from about 0.005 g/L to about 0.2 g/L, preferably about 0.01 to about 0.02 g/L.

The probiotic strain *Bacillus coagulans* may be co-administered with the vitamin B2. Hence, the
25 invention also relates to vitamin B2 for uses in increasing the abundance of *Bacillus coagulans* in the intestine of an animal, preferably a human, wherein said uses comprise delivering both the vitamin B2 and *Bacillus coagulans* to the large intestine. This can be done, for example, if *Bacillus coagulans* is not yet colonizing the intestine, or is present only in low numbers. *Bacillus coagulans* BC30 is preferred for co-administration.

30 For co-administration, including simultaneous administration/ delivery/ consumption, the vitamin and the probiotic can be in the same compartment, or in separate compartments. For example, the vitamin and the bacteria can be in the same tablet/ pill, or in the same sachet. Alternatively, the vitamin and the bacteria can be in separate tablets/ pills, or in different sachets. In the latter

case, an administration/ delivery/ consumption within 24 hours is still considered a co-administration.

Bacillus coagulans is known to improve the digestive health and to positively modulate the immune system. A number of studies have shown that the population of *Bacillus coagulans* in the gut microbiome is decreased when an animal, preferably a human is suffering from a particular disease/ adverse condition as compared to the population present in the animal not suffering from that particular disease/ adverse condition. However, none of these studies have suggested a method of how to increase the population of *Bacillus coagulans*, thus alleviating at least one of the symptoms of the disease/ adverse condition. It has been found, in accordance with this invention, that direct delivery of vitamin B2 to the large intestine of an animal, preferably a human, can increase the population of *Bacillus coagulans* in the large intestine.

In a preferred embodiment, the animal (including a human) is experiencing a condition selected from the group consisting of: irritable bowel syndrome (IBS), functional intestinal gas symptoms, diarrhea, abdominal pain and bloating, upper respiratory symptoms, and airway infections.

Preferably, the *Bacillus coagulans* to be boosted is *Bacillus coagulans* BC30. Thus, in one embodiment, the vitamin B2 is for use in increasing the abundance of *Bacillus coagulans* BC30 in the large intestine of an animal.

In another aspect, the present invention relates to an oral formulation comprising vitamin B2 and *Bacillus coagulans*. Preferably, the oral formulation is a delayed-release formulation.

In yet another aspect, the present invention relates to a method of increasing the abundance of *Bacillus coagulans* in the intestine, preferably the large intestine, comprising administering to the animal an effective dose of vitamin B2. The method is for improving intestinal health in an animal, including a human, wherein said improvement comprises increasing the abundance of *Bacillus coagulans*, preferably *Bacillus coagulans* BC30, in the large intestine. Preferably, the animal is a human.

Preferably, the vitamin B2 is delivered directly to the large intestine. Delivery to the large intestine can be achieved by administering the vitamin B2 as a delayed-release formulation.

In one embodiment, *Bacillus coagulans* is co-administered with the vitamin B2. Preferably, an effective dose of *Bacillus coagulans* BC30 is co-administered.

The methods of the invention can be used to treat, prevent, and/or lessen the symptoms of irritable bowel syndrome (IBS), functional intestinal gas symptoms, diarrhea, abdominal pain and bloating, upper respiratory symptoms, and airway infections in an animal, including a human, in need thereof.

In a further aspect, the present invention relates to the use of vitamin B2 for increasing the abundance of *Bacillus coagulans* in the intestine of an animal, preferably a human, wherein said use comprises delivering the vitamin B2 to the large intestine. Preferably, the use comprises delivering/ administering the vitamin B2 to the large intestine by a delayed-release formulation. In one embodiment, the use comprises administering to the animal a formulation comprising a vitamin B2 dose of up to 200 mg/day, preferably 5-100 mg/day, more preferably 10-50 mg/day. The vitamin B2 may be co-administered with *Bacillus coagulans*. Preferably, the animal, including a human, is experiencing a condition selected from the group consisting of: irritable bowel syndrome, functional intestinal gas symptoms, diarrhea, abdominal pain and bloating, upper respiratory symptoms, and airway infections. Preferably, the *Bacillus coagulans* is *Bacillus coagulans* BC30.

A preferred way of (co-)administration is oral administration.

Definitions

As used throughout, the following definitions apply:

The term "vitamin B2", which is used interchangeably with "riboflavin", includes riboflavin and esters thereof, in particular riboflavin-5'-phosphate and other pharmaceutically acceptable forms.

To "increase the abundance" of *Bacillus coagulans* means to increase the level (or the amount, or number, or the population size) of *Bacillus coagulans* compared to the respective control (i.e., the level/ amount/ number/ population size of *Bacillus coagulans* without the addition of vitamin B2).

The term "intestine" (or "gut") as used herein refers to the portion of the gastrointestinal tract consisting of the small intestine and the large intestine. The "large intestine" (*intestinum crassum*) is the lower part of the gastrointestinal tract and is also referred to herein as "colon".

5 "Direct delivery" or "directly delivered" means that the vitamin is formulated in a manner such that the vitamin is not absorbed in the stomach and/or small intestine; rather the vitamin is made available in the distal intestinal tract, preferably the large intestine (colon), where it is available to the microbiome. The vitamin is not part of a person's usual daily nutritional requirements (generally obtained through diet and conventional vitamin supplementation), and is administered
10 in excess thereof. For human use, the preferred method according to the present invention is through a form which delays release until the large intestinal tract (colon) is reached. Alternatively, a large enough dose can be administered, so that only a portion of the administered vitamin is absorbed in the proximal small intestine, and the remainder, which is an effective dose, is available to the large intestinal tract; although not preferred, the latter method of delivery can be
15 used for humans as well.

A used herein, "delayed release" refers to the release of the vitamin and/or the probiotic at a time later than immediately after administration. Preferably, "delayed release" means delivery of the vitamin (and/or probiotic), upon oral administration, to the large intestine (colon) in a delayed
20 manner relative to an immediate release formulation.

An "enteric layer" or "enteric coating" is a layer surrounding a core, wherein the core comprises the active agent and the layer confers resistance to gastric juice.

25 "Prevent" can include lessening the risk of an adverse condition occurring, lessening the symptoms of an adverse condition, lessening the severity of an adverse condition, and prolonging the time for occurrence of an adverse condition.

"Oral formulation" means that the vitamin and/or probiotic is formulated for oral administration/
30 consumption.

"Co-administering" or "co-administration" means that the vitamin and/or the probiotic is delivered/ administered/ consumed simultaneously (i.e., together), or separately but within a time frame of

24 hours. The vitamin can be delivered/ administered/ consumed first; likewise, the probiotic can be delivered/ administered/ consumed first.

Doses

- 5 The vitamin B2 can be administered in an amount such that its local concentration in the colon is at least 0.001 g/L, preferably at least 0.01 g/L more preferably at 0.02 g/L. Preferred local concentrations in the colon range from about 0.001 g/L to about 0.5 g/L or from about 0.005 g/L to about 0.2 g/L, preferably about 0.01 to about 0.02 g/L. Specific dosages per day can range up to 200 mg/day, preferably 5-100 mg/day, more preferably from 10-50 mg/day.

10

The dosage of the probiotic can be up to 5×10^{10} cfu/day. Preferably, the dosage range of the probiotic is from 1×10^8 to 1×10^{10} cfu/day, more preferably from 1×10^9 to 5×10^{10} cfu/day.

Formulations

- 15 The vitamin (and, where the vitamin is co-administered with the probiotic, preferably also the probiotic) is preferably present in a formulation which allows the vitamin (and/or probiotic) to be available predominantly in the large intestine.

20 Oral formulations are preferred. Other formulations include non-oral routes, such as via suppositories or injections.

For human use, the preferred method is through a delayed-release form which delays delivery until the intestinal tract is reached. For non-human animals, a preferred delivery includes a method of administering a large enough dose so that only a portion of the vitamin delivered is absorbed in the stomach, and the remainder, which is an effective dose, is available to the intestinal tract; although not preferred, this method of delivery can be used for humans as well.

25

Delayed-release formulations are known in the art. Preferably, the delayed-release formulations have an enteric coating (also referred to as enteric layer).

30

In one embodiment of the present invention, the vitamin (and, where the vitamin is co-administered with the probiotic, preferably also the probiotic) is in a formulation comprising an enteric capsule, filled with a composition comprising the vitamin. The enteric capsule confers

resistance against the acidic environment of the stomach. For example, soft gel formulations may deliver the active agent in solution and yet offer advantages of solid dosage forms.

5 In another embodiment, the formulation is a tablet comprising (i) a core comprising the vitamin (and/or the probiotic), and (ii) a delayed-release coating such as an enteric coating. This may be a hard gel capsule.

10 Alternatively, a matrix-based delivery system can be used for direct colon delivery. Matrix based systems have no discrete layer of coating material but the active agent is more or less homogenously distributed within the matrix. Further, there are colon-release systems that embed the active agent in e.g. in a fiber matrix (enzyme-triggered) and an enteric coating on top.

The release of the drug may be delayed until the small intestine. In another embodiment, the release of the drug is delayed until the distal small intestine. In yet another, preferred embodiment, 15 the release of the drug is delayed until the colon (large intestine).

In a preferred embodiment for humans, the vitamin is formulated in a solid dosage form for oral administration. The formulation may be in the form of a capsule, pellet, bead, sphere, mini spheres, tablet, mini tablet, or granule, optionally coated with a delayed release coating that 20 prevents the release of the active agent before the small intestine, preferably before the colon.

Coating, or matrix materials for the delayed release of the vitamin and/or probiotic, in particular for targeted release in the ileum or the large intestine upon oral administration are known in the art. They can be subdivided into coating materials that disintegrate above a specific pH, coating 25 materials that disintegrate after a specific residence time in the gastrointestinal tract and coating materials that disintegrate due enzymatic triggers specific to the microflora of a specific region of the intestines. Coating materials from different categories are commonly used in combinations. Coating materials of the different categories for targeting to the large intestine have been reviewed for example in Bansal et al. (Polim. Med. 2014, 44, 2,109-118). In one embodiment of the present 30 invention, the delayed-release coating comprises at least one component selected from coating materials that disintegrate pH-dependently, coating materials that disintegrate time-dependently, coating materials that disintegrate due to enzymatic triggers in the intestinal environment (e.g., in the intestinal environment of the ileum and the large intestine), and combinations thereof.

Coating materials that disintegrate pH-dependently include polyvinyl acetate phthalate, cellulose acetate trimellitate, hydroxypropyl methylcellulose phthalate HP-50, HP-55 or HP-55S, cellulose acetate phthalate, shellac, hydroxypropyl methylcellulose acetate succinate (HPMCAS), poly(methacrylic acid, ethyl acrylate) 1:1 (Eudragit® L100-55, Eudragit® L30D-55),
5 poly(methacrylic acid, methyl methacrylate) 1:1 (Eudragit® L-100, Eudragit® L12.5), poly(methacrylic acid, methyl methacrylate) 1:2 (Eudragit® S-100, Eudragit® S12.5, and Eudragit® FS30D). Coating materials that disintegrate time-dependently include Eudragit® RL, Eudragit®RS, and ethylcellulose. Coating materials that disintegrate due to enzymatic triggers in the large intestinal environment include chondroitin sulfate, pectin, guar gum, chitosan, inulin,
10 lactulose, raffinose, stachyose, alginate, dextran, xanthan gum, locust bean gum, arabinogalactan, cyclodextrin, pullulan, carrageenan, scleroglucan, chitin, curdulan, levan, amylopectin, starch, amylose, resistant starch, and azo compounds being degraded by azo bonds splitting bacteria.

15 The following non-limiting examples are presented to better illustrate the invention.

EXAMPLES

The aim of this study was to investigate the effect of vitamin B2 on bacteria of the species *Bacillus coagulans* in the human gut microbiota.

20

Materials and Methods

Design of the batch-fermentation experiment (colon model)

A short-term batch-fermentation experiment was carried out by ProDigest, consisting of a colonic incubation of a representative dose of a selected vitamin with a representative bacterial inoculum
25 under simulated conditions for the proximal large intestine. In the current experiment, the bacterial inocula were derived from fresh fecal samples of six different healthy adult donors. Incubations were performed as described previously (Van den Abbeele, P.; Taminiau, B.; Pinheiro, I.; Duysburgh, C.; Jacobs, H.; Pijls, L.; Marzorati, M. Arabinoxyl-Oligosaccharides and Inulin Impact Inter-Individual Variation on Microbial Metabolism and Composition, Which Immunomodulates
30 Human Cells. J. Agric. Food Chem. 2018, 66, 1121–1130). At the start of the short-term colonic incubation, fresh fecal material from six healthy human donors was collected and upon preparation of an anaerobic fecal slurry, this slurry was inoculated at 10 vol% in a SHIME nutritional medium containing basal nutrients containing 3.5 g/L K₂HPO₄, 10.9 g/L KH₂PO₄, 2 g/L NaHCO₃ (Chem-lab NV, Zedelgem, Belgium), 2 g/L yeast extract, 2 g/L peptone (Oxoid,

Aalst, Belgium), 1 g/L mucin (Carl Roth, Karlsruhe, Germany), 0.5 g/L L-cysteine and 2 mL/L Tween80 (Sigma-Aldrich, Bornem, Belgium). All test ingredients (i.e., probiotic strain, vitamin B2) were also added to the SHIME medium. Furthermore, M-SHIME® technology was incorporated in the current experiment by adding mucin-covered microcosms (modeling the mucus of the colon) to the incubations as described previously (Van den Abbeele, P., et al. (2013). Butyrate-producing Clostridium cluster XIVa species specifically colonize mucins in an in vitro gut model. The ISME Journal 7(5), 949–961). Incubations were performed for 48h, at 37°C, under shaking (90 rpm) and anaerobic conditions.

The probiotic strain used in this experiment was *Bacillus coagulans* BC30 (also known as *Bacillus coagulans* GBI-30, 6086), and was purchased from Schiff (Digestive Advantage, Daily Probiotic Capsule). The probiotic was isolated from the capsule and added as an overnight grown pure culture. The probiotic *Bacillus coagulans* strain was added to the colon model containing the donor samples alone or in combination with vitamin B2 (see Table 1), and the abundance of the added strain was analyzed after incubation. Experiments were performed in single repetition.

Table 1: Experiment layout

Test ingredient	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6
<i>Bacillus coagulans</i> BC30 only (Control)	x	x	x	x	x	x
<i>Bacillus coagulans</i> BC30 + Vitamin B2	x	x	x	x	x	x

The examined *Bacillus coagulans* BC30 strain was added to the SHIME medium at a concentration of 1×10^9 CFU. Vitamin B2 (Riboflavin TG, DSM) was added at a concentration of 0.01667 g/L which translates into a 10 mg dose, given that the colon volume is about 600 ml.

Analysis of the *Bacillus coagulans* BC30 level

qPCR was performed on ComK gene (NCBI reference CP026649) to monitor the level of the supplemented probiotic strain *Bacillus coagulans* BC30 within the gut microbiome. Primers were derived from a Chinese culture collection (bnbio.com). Samples were analyzed from the mucus environment following 48h of incubation.

Statistics

Statistical analysis was performed to investigate the average effect of the test products. For this purpose, the average over the six donors was calculated for each endpoint. Paired t-tests were conducted to evaluate the potential effect of the test products as compared to the control as well as to compare the different test products with each other. For the microbial community composition, statistical tests were performed on the log-transformed data (to make them normally distributed). Differences were considered statistically significant if the p-value was less than 0.05.

Results

Supplementation of vitamin B2 increased the level of *Bacillus coagulans* BC30

As shown in Figure 1, around three times as many copies of the *Bacillus coagulans* BC30 specific ComK gene were detected in the mucus environment by qPCR analysis when vitamin B2 was co-supplemented with *Bacillus coagulans* BC30. Hence, the addition of vitamin B2 significantly increased the levels of *Bacillus coagulans* BC30 in the colon model, as compared to the respective control.

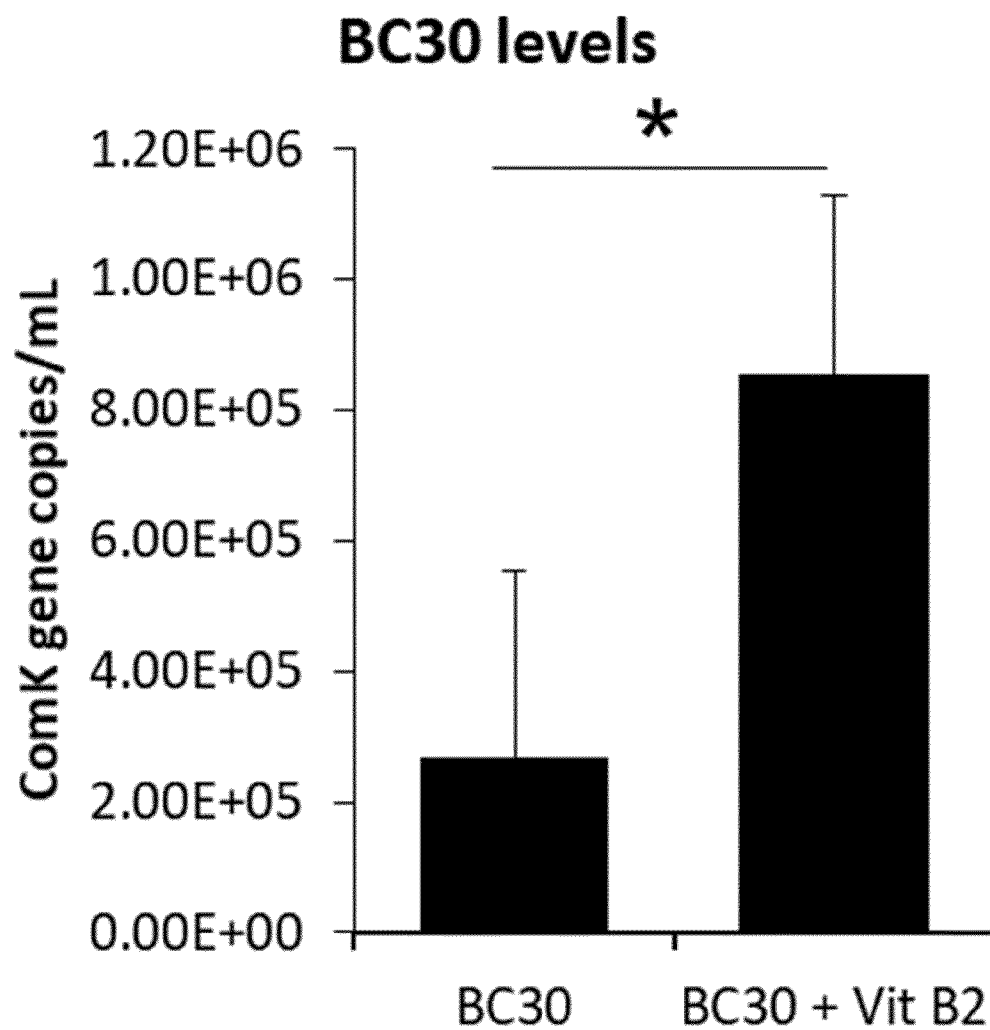
Claims

- 5 1. Vitamin B2 for use in increasing the abundance of *Bacillus coagulans* in the intestine of an animal, preferably a human, wherein said use comprises delivering the vitamin B2 to the large intestine.
2. Vitamin B2 for the use according to claim 1, wherein the vitamin B2 is delivered to the large intestine by a delayed-release formulation.
- 10 3. Vitamin B2 for the use according to claim 1 or 2, wherein said use comprises administering to the animal a formulation comprising a vitamin B2 dose of up to 200 mg/day, preferably 5-100 mg/day, more preferably 10-50 mg/day.
- 15 4. Vitamin B2 for the use according to any one of claims 1-3, wherein the vitamin B2 is co-administered with *Bacillus coagulans*.
- 20 5. Vitamin B2 for the use according to any one of claims 1-4, wherein the animal, including a human, is experiencing a condition selected from the group consisting of: irritable bowel syndrome, functional intestinal gas symptoms, diarrhea, abdominal pain and bloating, upper respiratory symptoms, airway infections.
6. Vitamin B2 for the use according to any one of claims 1-5, wherein the *Bacillus coagulans* is *Bacillus coagulans* BC30.
- 25 7. An oral formulation comprising vitamin B2 and *Bacillus coagulans*.
8. The oral formulation according to claim 7, which is a delayed-release formulation.
- 30 9. A method of increasing the abundance of *Bacillus coagulans* in the intestine of an animal, preferably a human, comprising administering to the animal a formulation comprising an effective dose of vitamin B2.
10. The method according to claim 9, wherein the animal is a human and the vitamin B2 is delivered to the large intestine.

11. The method according to claim 9 or 10, wherein the vitamin B2 is delivered by a delayed-release formulation.
- 5 12. The method according to any one of claims 9-11, wherein the vitamin B2 is co-administered with *Bacillus coagulans*.
- 10 13. The method according to any one of claims 9-12, which is a method of treating, preventing, or lessening the symptoms of irritable bowel syndrome, functional intestinal gas symptoms, diarrhea, abdominal pain and bloating, upper respiratory symptoms, or airway infections in an animal, including a human, in need thereof.
14. The method according to any one of claims 9-13, wherein the *Bacillus coagulans* is *Bacillus coagulans* BC30.

1/1

Fig. 1



INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER INV. A61K31/525 A61K35/742 A61P1/00 A61P1/12 A61P11/00 ADD.		
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) A61P A61K		
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 practicable, search terms used) EPO-Internal		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	page 7 – page 8 -----	1-6, 8-14
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Y	page 4, paragraph 4 page 5, paragraph 1 page 12, paragraph 4 -----	1-6, 8-14
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☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent 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		Date of mailing of the international search report
3 August 2023		10/08/2023
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Healy, Cathal

INTERNATIONAL SEARCH REPORT

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