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(54) Title: VITAMIN B1 FOR USE IN IMPROVING GUT HEALTH

(57) Abstract: The present invention relates to vitamin B1 (thiamine) for use in improving gut health in animals and humans. In particular, it was found that vitamin B1, upon delivery to the large intestine, increases the adhesion of the beneficial bacteria of the species *Bacillus coagulans* in the intestinal tract.



VITAMIN B1 FOR USE IN IMPROVING GUT HEALTH

FIELD OF THE INVENTION

The present invention relates to vitamin B1 (thiamin) for use in improving gut health in animals
5 and humans. It was found that vitamin B1, upon delivery to the large intestine, increases the
adhesion 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-
20 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.,
25 *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 Bacterial adhesion is a process that allows bacteria to attach or adhere to other cells and surfaces.
Adhesion is an important step in colonization of a new host or environment. Indeed, adherence is
an essential process for the survival and proliferation of probiotic microorganisms in the gut
(Kadlec R et al., The effect of prebiotics on adherence of probiotics (2014)).

Adhesive probiotics can prevent the subsequent attachment of pathogens, referred to as competitive exclusion. The attachment of probiotics to the intestinal mucosa can have a potential protective role against enteropathogens through competition for host cell binding sites. In addition, the adhesion ability of probiotic bacteria increases their opportunity to interact with the host, resulting in a temporary colonization. Higher adherence also results in prolonging their transit time in the gut, enabling them to proliferate and exert their beneficial effects. For instance, this temporary colonisation favours the local action of metabolites produced by probiotics (such a short-chain fatty acids, SCFAs), as well as immunomodulatory effects by bacterial surface-located molecules which act as ligands for host receptors in the intestinal epithelium inducing signalling pathways (Monteagudo-Mera A, Adhesion mechanisms mediated by probiotics and prebiotics and their potential impact on human health (2019)).

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. One way to boost the beneficial bacteria would be by enhancing their adhesion, which would in turn provide them with enough time to proliferate in the colon. In particular, it would be desirable to increase the adhesion of *Bacillus coagulans* in the intestine in order to enhance wellness, improve digestive health, and support the immune system.

SUMMARY OF THE INVENTION

The present invention relates to the following items:

- 1) Vitamin B1 for use in increasing the adhesion of *Bacillus coagulans* in the intestine of an animal, preferably a human, wherein said use comprises delivering the vitamin B1 to the large intestine.
- 2) Vitamin B1 for the use according to item 1, wherein the vitamin B1 is delivered to the large intestine by a delayed-release formulation.
- 3) Vitamin B1 for the use according to item 1 or 2, wherein said use comprises administering to the animal a formulation comprising a vitamin B1 dose of up to 20 mg/day, preferably 0.5-10 mg/day, more preferably 1-5 mg/day.
- 4) Vitamin B1 for the use according to any one of items 1-3, wherein the vitamin B1 is co-administered with *Bacillus coagulans*.

- 5) Vitamin B1 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, and airway infections.
- 5 6) Vitamin B1 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 B1 and *Bacillus coagulans*.
- 8) The oral formulation according to item 7 which is a delayed-release formulation
- 9) A method of increasing the adhesion 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 B1.
- 10 10) The method according to item 9, wherein the animal is a human and the vitamin B1 is delivered to the large intestine.
- 11) The method according to item 9 or 10, wherein the vitamin B1 is delivered by a delayed-release formulation.
- 15 12) The method according to any one of claims 9-11, wherein *Bacillus coagulans* is co-administered with vitamin B1.
- 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, or airway infections in an animal, including a human, in need thereof.
- 20 14) The method according to any one of items 9-13, wherein the *Bacillus coagulans* is *Bacillus coagulans* BC30.

25 BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the adhesion of *Bacillus coagulans* BC30 to Caco-2 monolayers, alone or in the presence of different vitamins, as determined by flow cytometry. 5×10^7 bacteria were added to the Caco-2 cells in the absence (control) or in the presence of vitamins B1, B2, B3, B9, C, D3 or Vitamin Mix (mix of vitamins B2, B3, B5, B6, B9 and C) and incubated for 1h at 37°C. The number of bacteria adhered to the cells, in the medium and in the washing buffer was determined by flow cytometry. % adhesion was calculated as $\frac{\text{adhered bacteria}}{\text{adhered bacteria} + \text{bacteria in the medium} + \text{bacteria in the wash}} \times 100$. Bars represent mean $\pm$ SEM of triplicates. Statistically significant differences between the control and treatments are indicated with '*' ($p < 0.05$).

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DETAILED DESCRIPTION OF THE INVENTION

Bacillus coagulans is a bacterial strain known for its beneficial effects on human health, in particular gut health. The present inventors have found that vitamin B1 can boost the adhesion capabilities of *Bacillus coagulans*, ultimately leading to an increase of *Bacillus coagulans* levels in the gut.

Hence, in a first aspect, the present invention relates to vitamin B1 for use in improving gut health in an animal. Said improvement comprises or consists of increasing the adhesion of *Bacillus coagulans* in the intestine of said animal. Specifically, the vitamin B1 is for use in increasing the adhesion of *Bacillus coagulans* in the large intestine (colon) of an animal, and preferably, said use comprises delivering the vitamin B1 to the large intestine. Preferably, the animal is a human.

Vitamin B1 (also known as thiamine or thiamin) is one of the water-soluble B vitamins. Vitamin B1 is naturally present in some foods, added to some food products, and available as a dietary supplement. This vitamin plays a critical role in energy metabolism and, therefore, in the growth, development, and function of cells. Vitamin B1 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.

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 *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.

To achieve an increase of the adhesion of *Bacillus coagulans* in the large intestine, the vitamin B1 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 B1 in a delayed-release formulation. Oral administration is preferred.

5

Preferably, the vitamin B1 dose administered to the animal is up to 20 mg/day, preferably 0.5-10 mg/day, more preferably 1-5 mg/day. In one embodiment, vitamin B1 is administered in an amount such that its local concentration in the colon is at least 0.0001 g/L, preferably at least 0.001 g/L more preferably at 0.002 g/L. Preferred local concentrations in the colon range from about 0.0001 g/L to about 0.05 g/L or from about 0.0005 g/L to about 0.02 g/L, preferably about 0.001 to about 0.002 g/L.

10

The probiotic strain *Bacillus coagulans* may be co-administered with the vitamin B1. Hence, the invention also relates to vitamin B1 for uses in increasing the adhesion of *Bacillus coagulans* in the intestine of an animal, preferably a human, wherein said uses comprise delivering both the vitamin B1 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.

15

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.

20

25

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 adherence of *Bacillus coagulans*, thus alleviating at least one of the symptoms of the disease/ adverse condition.

30

It has been found, in accordance with this invention, that direct delivery of vitamin B1 to the large intestine of an animal, preferably a human, can increase the adhesion of *Bacillus coagulans* to the colon epithelium and, by doing so, provide it enough time to proliferate in the colon and increase its population in the colon.

5

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.

10 Preferably, the *Bacillus coagulans* to be boosted is *Bacillus coagulans* BC30. Thus, in one embodiment, the vitamin B1 is for use in increasing the adhesion of *Bacillus coagulans* BC30 in the large intestine of an animal.

In another aspect, the present invention relates to an oral formulation comprising vitamin B1 and
15 *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 adhesion of *Bacillus coagulans* in the intestine, preferably the large intestine, comprising administering to the animal an effective dose of vitamin B1. The method is for improving intestinal health in an animal,
20 including a human, wherein said improvement comprises increasing the adhesion of *Bacillus coagulans*, preferably *Bacillus coagulans* BC30, in the large intestine. Preferably, the animal is a human.

Preferably, the vitamin B1 is delivered directly to the large intestine. Delivery to the large intestine
25 can be achieved by administering the vitamin B1 as a delayed-release formulation.

In one embodiment, *Bacillus coagulans* is co-administered with the vitamin B1. Preferably, an effective dose of *Bacillus coagulans* BC30 is co-administered.

30 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 B1 for increasing the adherence of *Bacillus coagulans* in the intestine of an animal, preferably a human, wherein said use comprises delivering the vitamin B1 to the large intestine. Preferably, the use comprises delivering/ administering the vitamin B1 to the large intestine by a delayed-release formulation. In one embodiment, the use comprises administering to the animal a formulation comprising a vitamin B1 dose of up to 20 mg/day, preferably 0.5-10 mg/day, more preferably 1-5 mg/day. The vitamin B1 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 B1" is used interchangeably with "thiamin(e)" and "aneurine", and also comprises pharmaceutically acceptable forms thereof.

To "increase the adhesion", or to "increase the adhesion ability/capability" of *Bacillus coagulans* means to increase the number (or amount, or percentage) of *Bacillus coagulans* cells which are able to adhere to colon epithelial cells, compared to the respective control (i.e., the number/ amount/ percentage of *Bacillus coagulans* cells adhering to the colon epithelial cells without the addition of vitamin B1).

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".

"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 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 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 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.

"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/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

The Vitamin B1 can be administered in an amount such that its local concentration in the colon is at least 0.0001 g/L, preferably at least 0.001 g/L more preferably at 0.002 g/L. Preferred local concentrations in the colon range from about 0.0001 g/L to about 0.05 g/L or from about 0.0005 g/L to about 0.02 g/L, preferably about 0.001 to about 0.002 g/L. Specific dosages per day can range up to 20 mg/day, preferably 0.5-10 mg/day, more preferably from 1-5 mg/day.

The dosage of the probiotic can be up to $5E+10$ cfu/day. Preferably, the dosage range of the probiotic is from $1E+08$ to $1E+10$ cfu/day, more preferably from $1E+09$ to $5E+10$ cfu/day.

5 Formulations

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.

10 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
15 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.

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

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
25 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.

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
30 a hard gel capsule.

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, 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 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 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 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), 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,

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.

5

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 B1 on the ability of bacteria of the species *Bacillus coagulans* to adhere to the colon epithelium.

10

Design of the experiment

Cellular model

Caco-2 cells are epithelial cells isolated from colon tissue. Briefly, Caco-2 cells (HTB-37; American Type Culture Collection) were seeded at 2.5×10^5 cells in 24-wells coated with 0.1% gelatin. Cells were grown for 14 days in complete medium (Dulbecco's Modified Eagle Medium (DMEM) supplemented with 20% heat-inactivated fetal bovine serum (FBS), 10 mM HEPES and 1X antibiotic-antimycotic) with 3 medium changes/week. 1 to 2 days before the experiment, medium was changed to antibiotic-antimycotic free complete medium. The day of the experiment, cells were incubated in DMEM supplemented with 1% heat-inactivated FBS in presence or absence of the test products (probiotic strain, vitamins). After 1h incubation, cells were washed and lysed. Medium, wash steps and cell lysates were further diluted in PBS and analyzed by flow cytometry (BD FACSVerse™, BD Biosciences) upon staining with SYTO24 and propidium iodide.

20

Test products

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). Purity of the bacterial strain was confirmed by 16S rRNA sequencing. For the adhesion assays, the content of the capsule was administered directly to an Erlenmeyer containing TSB (tryptone soya broth) (Oxoid, Thermo Fisher Scientific), and aerobically incubated at 37°C under constant shaking for 24h or 48h. Bacteria were added to the cells at 5×10^7 cfu/mL.

30

Vitamins B1, B2, B5, B6, B9, C, and D3 were obtained from DSM Nutritional Products; while vitamin B3 was purchased from Sigma-Aldrich as nicotinic acid (N4126). Vitamins B1, B2, B3,

B9, C, and D3 were tested as individual products in the adhesion assay; in addition, a mix of vitamins B2, B3, B5, B6, B9 and C (Vitamin Mix) was tested. The concentrations used are shown in Table 1.

5 **Table 1:** List of test products and *in vitro* dosage tested in the adhesion experiment.

	Vitamin	Form	Dose (mg/mL)
Single vitamins	B1	Thiamine	0,001833
	B2	Riboflavin TG	0,016667
	B3	Nicotinic acid	0,008333
	B9	Folic acid	0,000667
	C	Ascorbic acid granular powder	0,333333
	D3	Cholecalciferol	0,000025
Vitamin mix	B2	Riboflavin TG	0,016667
	B3	Nicotinic acid	0,008333
	B5	Panthenic acid	0,008333
	B6	Pyridoxine Hydrochloride	0,002833
	B9	Folic acid	0,000667
	C	Ascorbic acid granular powder	0,333333

Data processing and statistical analysis

% adhesion was calculated as counts adhered bacteria/(counts adhered bacteria + counts bacteria in medium + counts bacteria in washing steps) x 100. Statistical analysis was performed using the GraphPad Prism 8.3.0 software. Differences in % adhesion between the control and treatments were determined using an ordinary one-way ANOVA with Dunnett's multiple comparisons test and are represented by (*). (*) = p<0.05.

Results

15 **Supplementation of vitamin B1 increased the adhesion of *Bacillus coagulans* BC30**

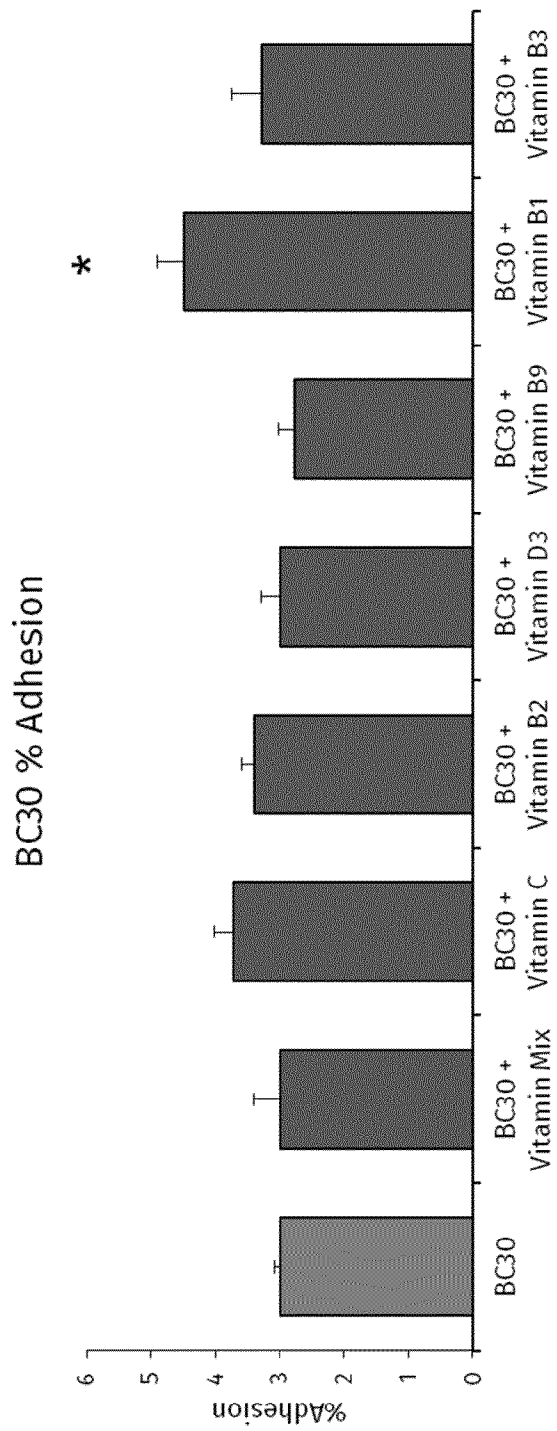
As shown in Figure 1, adhesion of *Bacillus coagulans* BC30 to intestinal epithelial cells was significantly increased upon addition of vitamin B1 compared to the respective control. None of the other tested vitamins significantly increased the adhesion of BC30 to intestinal epithelial cells compared to the respective control.

Claims

1. Vitamin B1 for use in increasing the adhesion of *Bacillus coagulans* in the intestine of an animal, preferably a human, wherein said use comprises delivering the vitamin B1 to the large intestine.
2. Vitamin B1 for the use according to claim 1, wherein the vitamin B1 is delivered to the large intestine by a delayed-release formulation.
3. Vitamin B1 for the use according to claim 1 or 2, wherein said use comprises administering to the animal a formulation comprising a vitamin B1 dose of up to 20 mg/day, preferably 0.5-10 mg/day, more preferably 1-5 mg/day.
4. Vitamin B1 for the use according to any one of claims 1-3, wherein the vitamin B1 is co-administered with *Bacillus coagulans*.
5. Vitamin B1 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, and airway infections.
6. Vitamin B1 for the use according to any one of claims 1-5, wherein the *Bacillus coagulans* is *Bacillus coagulans* BC30.
7. An oral formulation comprising vitamin B1 and *Bacillus coagulans*.
8. The oral formulation according to claim 7, which is a delayed-release formulation.
9. A method of increasing the adhesion 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 B1.
10. The method according to claim 9, wherein the animal is a human and the vitamin B1 is delivered to the large intestine.

11. The method according to claim 9 or 10, wherein the vitamin B1 is delivered by a delayed-release formulation.
- 5 12. The method according to any one of claims 9-11, wherein *Bacillus coagulans* is co-administered with the vitamin B1.
- 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.

Fig. 1



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2023/065383**A. CLASSIFICATION OF SUBJECT MATTER**INV. **A61K31/51 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)

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 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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X	US 11 235 008 B2 (FARMER SEAN [US]; KELLER DAVID [US] ET AL.) 1 February 2022 (2022-02-01) column 4, paragraph 4 column 5, paragraph 1 column 12, paragraph 4 ----- --/--	7



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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Date of the actual completion of the international search

4 August 2023

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2023/065383

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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