

USE OF MICROORGANISMS TO IMPROVE VACCINE EFFICACY

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 63/166,878, filed March 26, 2021, the contents of which are hereby incorporated by reference
5 in the entirety for all purposes.

BACKGROUND OF THE INVENTION

[0002] In recent years, viral and bacterial infection is becoming more prevalent worldwide and presents a serious public health threat. For example, the Coronavirus-2019 (COVID-19) global pandemic of a respiratory disease caused by severe acute respiratory syndrome
10 coronavirus 2 (SARS-CoV-2) has affected nearly 444 million people worldwide, including over 6 million deaths, and is exacerbated by a large burden of asymptomatic carriers as well as a lack of thoroughly tested, proven safe and effective vaccines. Several promising therapeutic agents are currently undergoing active investigation and development for prophylactic or therapeutic use in the treatment for COVID-19 to prevent or ameliorate its
15 damaging effects to the afflicted patients, while in the meantime experimental vaccines are widely distributed to the general population.

[0003] Accordingly, there exists an urgent need for new and meaningful methods to facilitate vaccination efforts by way of enhancing vaccine safety and efficacy to achieve reduction or elimination of viral and bacterial infections as well as to lessen or eliminate their
20 associated effects. The purpose of this study is to identify gut microbial species and microbial metabolites that can potentially enhance the beneficial effects of vaccination among recipients. Direct supplementation of these beneficial gut microbial species or direct supplementation of the beneficial microbial metabolites is a potentially effective means to improve a patient's immunity against an infectious pathogen upon vaccination against
25 infections including COVID-19, thus preventing the development of such infectious diseases and/or reducing the severity of such diseases. The present invention fulfills this and other related needs by identifying beneficial gut microorganisms as well as their metabolites in the prevention of diseases and conditions caused by such infections.

BRIEF SUMMARY OF THE INVENTION

[0004] The present inventors discovered in their studies the certain gut microbial species and their metabolites can enhance the benefits of vaccines against infectious pathogens (*e.g.*, viral or bacterial pathogens such as SARS-CoV-2) including improving the efficacy and safety of the vaccines, thus provide important utility in the prevention and eradication of such diseases. The microorganisms and metabolites so identified now serve to provide new methods and compositions for enhancing or improving an individuals' immunity from vaccination against infectious diseases such as COVID-19.

[0005] In a first aspect, the present invention provides a composition that is useful for improving vaccine efficacy and safety, for reducing inflammation, and for increasing antibody response induced by the vaccine, thereby enhancing the overall effectiveness of a vaccine in a subject. The composition is typically administered to a subject at approximately the same time of vaccination. The composition comprises (1) an effective amount of *bifidobacteria adolescentis*, *bifidobacteria bifidum*, and *bifidobacteria longum* or their metabolites; and (2) a physiologically acceptable excipient. In some embodiments, the composition further comprises an effective amount of xylooligosaccharides, galactooligosaccharides, and resistant dextrin (soluble corn fiber). In some embodiments, the composition consists of or consists essentially of (1) an effective amount of *bifidobacteria adolescentis*, *bifidobacteria bifidum*, and *bifidobacteria longum*; and (2) one or more physiologically acceptable excipients. In some embodiments, the composition consists of or consists essentially of (1) an effective amount of *bifidobacteria adolescentis*, *bifidobacteria bifidum*, and *bifidobacteria longum*, xylooligosaccharides, galactooligosaccharides, and resistant dextrin (soluble corn fiber); and (2) one or more physiologically acceptable excipients. In some embodiments, included in the composition is the medium in which *bifidobacteria adolescentis*, *bifidobacteria bifidum*, and *bifidobacteria longum* have been cultured and therefore contains an effective amount of their metabolites.

[0006] In some embodiments, *bifidobacteria adolescentis*, *bifidobacteria bifidum*, and *bifidobacteria longum* are present in the composition in the weight ratio of about (0.57-3.56): about 1: about (0.21-2.36). In some embodiments, *bifidobacteria adolescentis*, *bifidobacteria bifidum*, and *bifidobacteria longum* are present in the composition in the weight ratio of about (0.75-1): about 1: about (0.75-1) for an adult subject, or about (0.57-3.09):about 1: about

(0.21-1.7) for a child subject. In some embodiments, xylooligosaccharides, galactooligosaccharides, and resistant dextrin (soluble corn fiber) are present in the composition in the weight ratio of about (0.25-0.5): about (2-4): about (0.5-0.75). In some embodiments, the composition is formulated for oral ingestion. In some embodiments, the composition is formulated in a daily dosage comprising *bifidobacteria adolescentis*, *bifidobacteria bifidum*, and *bifidobacteria longum* in a combined range of about 10^6 to about 10^{12} colony-forming unit (CFU). In some embodiments, the composition is formulated in a daily dosage comprising xylooligosaccharides, galactooligosaccharides, and resistant dextrin (soluble corn fiber) in a combined range of about 0.01 to about 12 grams, for example, about 0.5 to about 6 grams, about 1 to about 3 grams, or about 1.2 to about 1.5 grams, with higher levels more suitable for use in combination with food especially when mixed in a food item. In some embodiments, the composition is formulated in the form of a food or beverage item, for example, in a daily dosage comprising xylooligosaccharides, galactooligosaccharides, and resistant dextrin (soluble corn fiber) in the amount of about 0.1 to about 12 grams. In some embodiments, the composition may further includes one or more agents known to effectively suppress SARS-CoV-2 and facilitate patient recovery from COVID-19 (e.g., ivermectin, hydroxychloroquine, fluvoxamine/fluoxetine, Zinc, vitamin C, vitamin D, quercetin, melatonin, azithromycin, doxycycline, or any combination thereof).

[0007] In the second aspect, the present invention provides a method for improving vaccine efficacy and safety, for reducing inflammation, and for increasing antibody response induced by the vaccine, thereby enhancing the overall effectiveness of a vaccine in a subject. The method includes the step of administering to an individual an effective amount of a composition described above or herein, typically administered at approximately the same time of vaccination. In some embodiments, the composition is orally administered to the subject. In some embodiments, the subject is administered one single composition comprising an effective amount of *bifidobacteria adolescentis*, *bifidobacteria bifidum*, *bifidobacteria longum*, xylooligosaccharides, galactooligosaccharides, and resistant dextrin (soluble corn fiber). In some embodiments, the subject is administered two or more compositions each comprising one or more of *bifidobacteria adolescentis*, *bifidobacteria bifidum*, *bifidobacteria longum*, xylooligosaccharides, galactooligosaccharides, and resistant dextrin (soluble corn fiber), together providing a combined effective amount of these

individual components. In some embodiments, the subject is not diagnosed with COVID-19 but may have had exposure to SARS-CoV-2 virus and therefore may be at risk of becoming infected. In some embodiments, the subject has received a vaccine for COVID-19 within the past 24 or 48 hours. In some embodiments, the subject is to receive a vaccine for COVID-19 within the next 24 or 48 hours. Thus, in some cases the method may further include a step of vaccinating the subject just prior or after administering to the subject the composition of this invention, *e.g.*, within 1 or 2 days, preferably on the same day. In some embodiments, especially when the subject is seeking prophylaxis for COVID-19, the method further includes administering to the subject one or more agents known to effectively suppress SARS-CoV2 and facilitate patient recovery from COVID-19 (*e.g.*, ivermectin, hydroxychloroquine, fluvoxamine or fluoxetine, Zinc, vitamin C, vitamin D, quercetin, melatonin, azithromycin, doxycycline, or any combination thereof), which may be in the same composition or in one or more separate compositions. In some embodiments, the vaccine is comprises a whole virus (*e.g.*, heat-inactivated or otherwise attenuated), a viral protein, a viral vector, or a nucleic acid (DNA or RNA) encoding a viral protein. For example, the vaccine is a Sinovac or Sinopharm vaccine, a Pfizer vaccine, a Moderna vaccine, a Janssen vaccine, or an AstraZeneca vaccine produced in March 2021 or later.

[0008] In a related aspect, the present invention provides a novel use of a composition for improving vaccine efficacy and safety, for reducing inflammation, and for increasing antibody response induced by the vaccine, thereby enhancing the overall effectiveness of a vaccine in a subject. The composition is typically administered to a subject at approximately the same time of vaccination. The composition, as described above or herein, includes an effective amount of *bifidobacteria adolescentis*, *bifidobacteria bifidum*, and *bifidobacteria longum*, optionally further including an effective amount of xylooligosaccharides, galactooligosaccharides, and resistant dextrin (soluble corn fiber), and is intended for administration to a subject, *e.g.*, by oral ingestion. In some embodiments, the subject is not diagnosed with COVID-19 but may have had an exposure to SARS-CoV-2 virus and is therefore at risk of becoming infected. In some embodiments, the subject has received a vaccine for COVID-19 within the past 24 or 48 hours. In some embodiments, the subject is to receive a vaccine for COVID-19 within the next 24 or 48 hours. In some embodiments, especially when the subject is seeking prophylaxis for COVID-19, the method further

- includes administering to the subject one or more agents known to effectively suppress SARS-CoV2 and facilitate patient recovery from COVID-19 (*e.g.*, ivermectin, Zinc, hydroxychloroquine, fluvoxamine/fluoxetine, vitamin C, vitamin D, quercetin, melatonin, azithromycin, doxycycline, or any combination thereof), which may be in the same
- 5 composition or in one or more separate compositions. In some embodiments, the vaccine is comprises a whole virus (*e.g.*, heat-inactivated or otherwise attenuated), a viral protein, a viral vector, or a nucleic acid (DNA or RNA) encoding a viral protein. For example, the vaccine is a Sinovac or Sinopharm vaccine, a Pfizer vaccine, a Moderna vaccine, an AstraZeneca vaccine, or a Janssen vaccine produced in March 2021 or later.
- 10 **[0009]** In a third aspect, the present invention provides a kit for improving vaccine efficacy and safety, for reducing inflammation, and for increasing antibody response induced by the vaccine, thereby enhancing the overall effectiveness of a vaccine in a subject. The kit includes at least two, possibly more, containers, each containing a composition comprising an effective amount of one or more active ingredients, which may be *bifidobacteria adolescentis*,
- 15 *bifidobacteria bifidum*, *bifidobacteria longum*, xylooligosaccharides, galactooligosaccharides, resistant dextrin (soluble corn fiber), or any combination thereof. For example, a first and second containers containing two different compositions, one comprising three of the above-named bifidobacterium species and the other comprising the three above-named metabolites. Typically, the compositions are in the form of a powder, liquid, paste, cream, tablet, or
- 20 capsule. The kit may further include an additional container containing one or more agents known to effectively suppress SARS-CoV-2 and facilitate patient recovery from COVID-19 (*e.g.*, ivermectin, fluvoxamine/fluoxetine, vitamin C, vitamin D, quercetin, melatonin, Zinc, azithromycin, doxycycline, hydroxychloroquine, or any combination thereof). Moreover, a user instruction manual may be included in the kit for the proper use of the kit, providing
- 25 information such as suitable subjects for treatment and time, dosing *etc.*

BRIEF DESCRIPTION OF THE DRAWINGS

- [0010]** **Figure 1.** Comparison of sCD40L, IL-1 β , TNF- α , IL-17A level between synbiotic and no synbiotic group after COVID-19 vaccination. 4 subjects had taken the synbiotic composition (synbiotic group) and 5 subjects had not taken the synbiotic composition prior to
- 30 COVID-19 vaccination (no synbiotic group). Blood samples were collected after these

subjects were vaccinated of 1st dose. The proinflammatory cytokines sCD40L, IL-1 β , TNF- α , IL-17A were quantified in the 2-days culture medium of PBMCs isolated from the blood samples. For all boxplots, the center was drawn through the median of the measurement while the lower and upper bounds of the box correspond to the first and third quantiles. n = 5 for no synbiotic group and n = 4 for synbiotic group. Statistical analysis was performed by Wilcoxon rank sum test. * $p < 0.05$, ** $p < 0.01$.

[0011] Figure 2. The proportion of total B cell subsets (CD45⁺CD19⁺) in PBMCs collected from subjects with (synbiotic group) or without (no synbiotic group) having the synbiotic composition before vaccination. For all boxplots, the center was drawn through the median of the measurement while the lower and upper bounds of the box correspond to the first and third quantiles. n = 5 for no synbiotic group and n = 4 for synbiotic group. Statistical analysis was performed by Wilcoxon rank sum test. * $p < 0.05$, ** $p < 0.01$.

[0012] Figure 3. The concentration of IgG and IgM antibodies to SARS-CoV-2 from subjects with (synbiotic group) or without (no synbiotic group) having the synbiotic composition before vaccination. For all boxplots, the center was drawn through the median of the measurement while the lower and upper bounds of the box correspond to the first and third quantiles. n = 5 for no synbiotic group and n = 4 for synbiotic group. Statistical analysis was performed by Wilcoxon rank sum test.

[0013] Figure 4. The total concentration of IgG antibodies to SARS-CoV-2 in the co-culture medium of PBMCs after co-cultured with 3 probiotic bacteria for 2 days. PBMCs isolated from one of the subjects in the no synbiotic group were co-cultured with the 3 probiotic bacteria (*B. adolescentis*, *B. bifidum*, and *B. longum*) simultaneously. PBMCs from the same subjects without co-culture with probiotic bacteria were used as control. Each group was measured in duplicate. Statistical analysis was performed by Student's t-test.

[0014] Figure 5. (A) The proportion of total B cells subsets (CD45⁺CD19⁺) in PBMCs (n=5), **(B)** total concentration of IgG antibodies to SARS-CoV-2 (n=2) and total concentration of IgM antibodies to SARS-CoV-2-S1 RBD (n=4) in the co-culture medium of PBMCs after co-cultured with metabolites from the 3 probiotics for 2 days. Supernatant of the co-culture medium of the 3 probiotic bacteria (*B. adolescentis*, *B. bifidum*, and *B. longum*) was co-cultured with PBMCs isolated from subjects after COVID-19 vaccination. PBMCs

from these subjects without co-culture with the supernatant was used as control. Each group was measured in duplicate. Statistical analysis was performed by Student's t-test.

DEFINITIONS

- [0015] As used herein, the term “**SARS-CoV-2** or severe acute respiratory syndrome coronavirus 2,” refers to the virus that causes Coronavirus Disease 2019 (COVID-19). It is also referred to as “COVID-19 virus.”
- [0016] The term “**xylooligosaccharides**” or XOS is used to refer to a group of oligosaccharides containing 2-6 molecules of xylose, whereas “**galactooligosaccharides**” or GOS are a group of oligosaccharides containing 2-9 molecules of galactose.
- [0017] The term “**inhibiting**” or “**inhibition**,” as used herein, refers to any detectable negative effect on a target biological process, such as RNA/protein expression of a target gene, the biological activity of a target protein, cellular signal transduction, cell proliferation, presence/level of an organism especially a micro-organism, any measurable biomarker, bio-parameter, or symptom in a subject, and the like. Typically, an inhibition is reflected in a decrease of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater in the target process (*e.g.*, a subject’s bodyweight, or the blood level of total cholesterol, or any measurable symptom or biomarker in a subject), or any one of the downstream parameters mentioned above, when compared to a control. “Inhibition” further includes a 100% reduction, *i.e.*, a complete elimination, prevention, or abolition of a target biological process or signal. The other relative terms such as “suppressing,” “suppression,” “reducing,” and “reduction” are used in a similar fashion in this disclosure to refer to decreases to different levels (*e.g.*, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater decrease compared to a control level) up to complete elimination of a target biological process or signal. On the other hand, terms such as “activate,” “activating,” “activation,” “increase,” “increasing,” “promote,” “promoting,” “enhance,” “enhancing,” or “enhancement” are used in this disclosure to encompass positive changes at different levels (*e.g.*, at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, or greater such as 3, 5, 8, 10, 20-fold increase compared to a control level in a target process, signal, or parameter).
- [0018] As used herein, the term “**treatment**” or “**treating**” includes both therapeutic and preventative measures taken to address the presence of a disease or condition or the risk of

developing such disease or condition at a later time. It encompasses therapeutic or preventive measures for alleviating ongoing symptoms, inhibiting or slowing disease progression, delaying of onset of symptoms, or eliminating or reducing side-effects caused by such disease or condition. A preventive measure in this context and its variations do not require 100%
5 elimination of the occurrence of an event; rather, they refer to a suppression or reduction in the likelihood or severity of such occurrence or a delay in such occurrence.

[0019] The term “**severity**” of a disease refers to the level and extent to which a disease progresses to cause detrimental effects on the well-being and health of a patient suffering from the disease, such as short-term and long-term physical, mental, and psychological
10 disability, up to and including death of the patient. Severity of a disease can be reflected in the nature and quantity of the necessary therapeutic and maintenance measures, the time duration required for patient recovery, the extent of possible recovery, the percentage of patient full recovery, the percentage of patients in need of long-term care, and mortality rate.

[0020] A “**patient**” or “**subject**” receiving the composition or treatment method of this invention is a human, including both adult and juvenile human, of any age, gender, and ethnic
15 background, who may not have been diagnosed with any particular disease or disorder (*e.g.*, have not had a positive nucleic acid and/or antibody test result for COVID-19) but is in need of enhancing immunity (*e.g.*, seeking vaccination to reduce or eliminate risk for a viral or bacterial infection). Typically, the patient or subject receiving treatment according to the
20 method of this invention to improve immunity is not otherwise in need of treatment by the same therapeutic agents. For example, if a subject is receiving the synbiotic composition according to the claimed method, the subject is not suffering from any disease that is known to be treated by the same therapeutic agents. Although a patient may be of any age, in some cases the patient is at least 40, 45, 50, 55, 60, 65, 70, 75, 80, or 85 years of age; in some cases,
25 a patient may be between 40 and 45 years old, or between 50 and 65 years of age, or between 65 and 85 years of age. A “child” subject is one under the age of 18 years, *e.g.*, about 5-17, 9 or 10-17, or 12-17 years old, whereas an “adult” subject is one who is 18 years or older.

[0021] The term “**effective amount**,” as used herein, refers to an amount that produces intended (*e.g.*, therapeutic) effects for which a substance is administered. The effects include
30 the prevention, correction, or inhibition of progression of the symptoms of a particular

disease/condition and related complications to any detectable extent, *e.g.*, one or more of the symptoms of a viral or bacterial infection and related disorder (*e.g.*, COVID-19), as well as improved vaccine safety and/or efficacy as reflected in any measurable reduction of adverse events and/or increase in the level of protection against the onset of a target disease (*e.g.*, COVID-19). The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*see, e.g.*, Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); and Pickar, *Dosage Calculations* (1999)).

5 [0022] The term “**about**” when used in reference to a given value denotes a range encompassing $\pm 10\%$ of the value.

[0023] A “**pharmaceutically acceptable**” or “**pharmacologically acceptable**” excipient is a substance that is not biologically harmful or otherwise undesirable, *i.e.*, the excipient may be administered to an individual along with a bioactive agent without causing any undesirable biological effects. Neither would the excipient interact in a deleterious manner with any of the components of the composition in which it is contained.

15 [0024] The term “**excipient**” refers to any essentially accessory substance that may be present in the finished dosage form of the composition of this invention. For example, the term “excipient” includes vehicles, binders, disintegrants, fillers (diluent), lubricants, glidants (flow enhancers), compression aids, colors, sweeteners, preservatives, suspending/dispersing agents, film formers/coatings, flavors and printing inks.

[0025] The term “**consisting essentially of**,” when used in the context of describing a composition containing an active ingredient or multiple active ingredients, refer to the fact that the composition does not contain other ingredients possessing any similar or relevant biological activity of the active ingredient(s) or capable of enhancing or suppressing the activity, whereas one or more inactive ingredients such as physiological or pharmaceutically acceptable excipients may be present in the composition. For example, a composition consisting essentially of active agents (for instance, a combination of *B. adolescentis*, *B. bifidum*, and *B. longum*) effective for enhancing the efficacy or safety of a COVID-19 vaccine is a composition that does not contain any other agents that may have any detectable positive or negative effect on the same target process (*e.g.*, COVID-19 prevention) or that

may increase or decrease to any measurable extent of the incidents of adverse events related to the vaccine among the receiving subjects.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

5 [0026] This invention describes a specific combination of bacteria (*B. adolescentis*, *B. bifidum*, and *B. longum*), optionally in further combination with certain prebiotics (xylooligosaccharides, galactooligosaccharides, resistant dextrin), for enhancing the safety and efficacy of vaccines, including vaccines for COVID-19. The practical use of the invention includes development and manufacturing of commercial food products or health
10 supplements, for example in the form of a powder, tablet, capsule, or liquid, which can be taken alone or added to food or beverages, especially in connection with vaccination efforts at or around the same time.

II. Pharmaceutical Compositions and Administration

[0027] The present invention provides pharmaceutical compositions comprising an
15 effective amount of *B. adolescentis*, *B. bifidum*, and *B. longum*, optionally in further combination with xylooligosaccharides, galactooligosaccharides, resistant dextrin for enhancing vaccine efficacy and/or safety in a person to reduce the risk of a disease involving viral or bacterial infection such as COVID-19. Pharmaceutical compositions of the invention are suitable for use in a variety of drug delivery systems. Suitable formulations for use in the
20 present invention are found in *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985). For a brief review of methods for drug delivery, see, Langer, *Science* **249**: 1527-1533 (1990).

[0028] The pharmaceutical compositions of the present invention can be administered by various routes, *e.g.*, systemic administration via oral ingestion or local delivery using a rectal
25 suppository. The preferred route of administering the pharmaceutical compositions is oral administration at daily doses of about 10^6 to about 10^{12} CFU for the combination of *B. adolescentis*, *B. bifidum*, and *B. longum*, at a weight ratio of about (0.57-3.56) to about 1, to about (0.21-2.36) in general, and preferably at a weight ratio of about (0.75-1) to about 1, to about (0.75-1) for adults and a weight ratio of about (0.57-3.09) to about 1, to about (0.21-1.7)
30 for children. Optionally, the prebiotic combination of xylooligosaccharides,

galactooligosaccharides, and resistant dextrin is further administered to the subject, either in one single composition or in multiple compositions, in daily doses of about 0.01 to about 6 grams, preferably about 1.2 to about 1.5 grams. When administered in the form of food consumption, the daily dosage may range from about 0.1 to about 12 grams. Typically, their weight ratio ranges from about (0.25-0.5) to about 1 to about (0.5-0.75) for both adults and children. The appropriate dose may be administered in a single daily dose or as divided doses presented at appropriate intervals, for example as two, three, four, or more subdoses per day. The duration of administration may range from about 2 weeks to about 4 weeks, *e.g.*, about 1 week to about 2 weeks, prior to or prior to vaccination, to at least about 2 weeks or at least about 4 weeks after vaccination, encompassing the day of vaccination.

[0029] For preparing pharmaceutical compositions containing the bifidobacteria combination and/or the prebiotics combination, one or more inert and pharmaceutically acceptable carriers are used. The pharmaceutical carrier can be either solid or liquid. Solid form preparations include, for example, powders, tablets, dispersible granules, capsules, cachets, and suppositories. A solid carrier can be one or more substances that can also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, or tablet disintegrating agents; it can also be an encapsulating material.

[0030] In powders, the carrier is generally a finely divided solid that is in a mixture with the finely divided active component, *e.g.*, the combination of *B. adolescentis*, *B. bifidum*, and *B. longum*, optionally further in combination of three prebiotics xylooligosaccharides, galactooligosaccharides, and resistant dextrin (Corn fiber). In tablets, the active ingredient is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

[0031] For preparing pharmaceutical compositions in the form of suppositories, a low-melting wax such as a mixture of fatty acid glycerides and cocoa butter is first melted and the active ingredient is dispersed therein by, for example, stirring. The molten homogeneous mixture is then poured into convenient-sized molds and allowed to cool and solidify.

[0032] Powders and tablets preferably contain between about 5% to about 100% by weight of the active ingredient(s) (*e.g.*, the combination of *B. adolescentis*, *B. bifidum*, and *B. longum*, optionally further in combination with three prebiotics xylooligosaccharides,

galactooligosaccharides, and resistant dextrin). Suitable carriers include, for example, magnesium carbonate, magnesium stearate, talc, lactose, sugar, pectin, dextrin, starch, tragacanth, methyl cellulose, sodium carboxymethyl cellulose, a low-melting wax, cocoa butter, and the like.

- 5 [0033] The pharmaceutical compositions can include the formulation of the active ingredient(s) *e.g.*, the combination of *B. adolescentis*, *B. bifidum*, and *B. longum*, optionally further in combination with three prebiotics xylooligosaccharides, galactooligosaccharides, and resistant dextrin, with encapsulating material as a carrier providing a capsule in which the active ingredient(s) (with or without other carriers) is surrounded by the carrier, such that the carrier is thus in association with the active ingredient(s). In a similar manner, sachets can also be included. Tablets, powders, sachets, and capsules can be used as solid dosage forms suitable for oral administration.

- [0034] Liquid pharmaceutical compositions include, for example, solutions suitable for oral administration or local delivery, suspensions, and emulsions suitable for oral administration.
- 15 Sterile water solutions of the active component (*e.g.*, *B. adolescentis*, *B. bifidum*, and *B. longum*, optionally further in combination with three prebiotics xylooligosaccharides, galactooligosaccharides, and resistant dextrin) or sterile solutions of the active component in solvents comprising water, buffered water, saline, PBS, ethanol, or propylene glycol are examples of liquid or semi-liquid compositions suitable for oral administration or local
- 20 delivery such as by rectal suppository. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents, detergents, and the like.

- [0035] Sterile solutions can be prepared by dissolving the active component (*e.g.*, the combination of *B. adolescentis*, *B. bifidum*, and *B. longum*, optionally further in combination with three prebiotics xylooligosaccharides, galactooligosaccharides, and resistant dextrin) in the desired solvent system, and then passing the resulting solution through a membrane filter to sterilize it or, alternatively, by dissolving the sterile active component in a previously sterilized solvent under sterile conditions. The resulting aqueous solutions may be packaged
- 30 for use as is, or lyophilized, the lyophilized preparation being combined with a sterile

aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, more preferably from 5 to 9, and most preferably from 7 to 8.

[0036] Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical
5 formulations should provide a quantity of an active agent sufficient to effectively enhance the efficacy of a vaccine and/or reduce or eliminate undesirable adverse effects of a vaccine.

III. Additional Therapeutic Agents

[0037] Additional known therapeutic agent or agents may be used in combination with an active agent such as *B. adolescentis*, *B. bifidum*, and *B. longum*, optionally further in
10 combination with prebiotics xylooligosaccharides, galactooligosaccharides, and resistant dextrin, in the practice of the present invention for the purpose of enhancing the safety and efficacy of a vaccine for the prevention or risk reduction of an infectious disease or associated disorder caused by a viral or bacterial infection such as COVID-19. In such applications, one or more of these previously known effective therapeutic agents can be
15 administered to patients concurrently with an effective amount of the active agent(s) either together in a single composition or separately in two or more different compositions.

[0038] For example, drugs and supplements that are known to be effective for use to prevent or treat COVID-19 include ivermectin, fluvoxamine/fluoxetine, vitamin C, vitamin D, melatonin, quercetin, Zinc, hydroxylchloroquine, and azithromycin. They may be used in
20 combination with the active agents (such as the combination of *B. adolescentis*, *B. bifidum*, and *B. longum* in an appropriate ratio among the three) of the present invention to enhance a COVID-19 vaccine's effectiveness and safety so as to reduce an individual's risk of SARS-CoV2 infection, reduce potential disease severity (including morbidity and mortality), facilitate recovery from the disease, as well as to improve safety profile of the vaccine/reduce
25 or eliminate adverse events stemmed from the vaccine. In particular, the combination of Zinc, hydroxylchloroquine, and azithromycin and the combination of ivermectin, fluvoxamine or fluoxetine, vitamin C, vitamin D, melatonin, quercetin, and Zinc have demonstrated high efficacy in both COVID prophylaxis and therapy. Thus, these known drug/supplement or nutraceutical combinations can be used in the method of this invention along with the active
30 components of the combination of *B. adolescentis*, *B. bifidum*, and *B. longum*, optionally

further with three prebiotics of xylooligosaccharides, galactooligosaccharides, and resistant dextrin.

IV. Kits

[0039] The invention also provides kits for improving vaccine efficacy and safety in an individual or for preventing an infectious disease/reducing severity of an infectious disease such as COVID-19 according to the method disclosed herein. The kits typically include a plurality of containers, each containing a composition comprising one or more of the bifidobacteria *B. adolescentis*, *B. bifidum*, and *B. longum*, or a combination thereof. Optionally, additional container(s) may be included in the kit providing composition(s) comprising one or more of prebiotics xylooligosaccharides, galactooligosaccharides, and resistant dextrin (soluble corn fiber), or any of their combinations. Further, additional agents or drugs that are known to be therapeutically effective for prevention and/or treatment of the disease, including for ameliorating the symptoms and reducing the severity of the disease, as well as for facilitating recovery from the disease (such as those described in the last section or otherwise known in the pertinent technical field) may be included in the kit. The plurality of containers of the kit each may contain a different active agent/drug or a distinct combination of two or more of the active agents or drugs. The kit may further include informational material providing instructions on how to dispense the pharmaceutical composition(s), including description of the type of patients who may be treated (*e.g.*, human patients who have been deemed as with high risk of contracting the disease, for example, due to having a high-risk profession such as medical care, but have not received a diagnosis of an infectious disease (*e.g.*, COVID-19) and are therefore seeking vaccination against the infectious disease or have just received the vaccine within the past one or two days), as well as the type of patients not to be included in the claimed method (*e.g.*, those have received a diagnosis of the infectious disease or have been diagnosed with a pre-existing condition that already requires the administration of the active components such as the combination of *B. adolescentis*, *B. bifidum*, and *B. longum*), the dosage, frequency, and manner of administration, and the like.

EXAMPLES

[0040] The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of non-critical parameters that could be changed or modified to yield essentially the same or similar results.

5 A SYNBIOTIC COMPOSITION

Selection of probiotic bacteria

[0041] Vaccines can stimulate the immune system to induce antibody formation, but a lack of beneficial bacteria can tip the balance to overwhelm our immune response leading to unwanted adverse effects. The present inventors discovered that *bifidobacterium*
 10 *adolescentis* (*B. adolescentis*), *bifidobacterium bifidum* (*B. bifidum*) and *bifidobacterium longum* (*B. longum*) (**Table 1**) together show positive correlation with desirable bacteria species that can potentially boost immunity. Such composition can enhance safety and efficacy of a COVID-19 vaccine.

Table 1 Probiotic bacteria to be included in the synbiotic composition

Bacterial species	NCBI:txid
<i>Bifidobacterium adolescentis</i>	1680
<i>Bifidobacterium bifidum</i>	1681
<i>Bifidobacterium longum</i>	216816

15

Ratio of probiotic microorganism

[0042] The ratio of *B. adolescentis*, *B. bifidum* and *B. longum* in the synbiotic composition is designed to be (0.57-3.56):1:(0.21-2.36), and preferably (0.75-1):1:(0.75-1), for adults. While for children, the ratio is (0.57-3.09):1:(0.21-1.7).

20 *Ratio of prebiotics*

[0043] A combination of 3 prebiotics can be added into the synbiotic composition. The 3 prebiotics in the synbiotic composition are beneficial for the growth of the probiotic microorganism, and could improve the delivery of the probiotic microorganism to the gut. As some of these prebiotics are fibers, they help with the coating process of probiotic bacteria
 25 during microencapsulation and provide a favorable environment for the probiotic bacteria to

survive during transit in the gastrointestinal tract. The ratio among the 3 prebiotics, xylooligosaccharides, galactooligosaccharides, and resistant dextrin (soluble corn fiber), is (0.25-0.5):(2-4):(0.5-0.75) for adult and children.

Formula of the synbiotic composition

5 **[0044]** The formula of synbiotic composition for enhancing safety and efficacy of COVID-19 vaccine comprises of 3 bifidobacterium (*Bifidobacterium adolescentis*, *Bifidobacterium bifidum* and *Bifidobacterium longum*) and 3 prebiotics (xylooligosaccharide, galactooligosaccharides, resistant dextrin (corn fiber)) with suggested dosage in **Table 2**. The 3 prebiotics are optional. A formula comprises of the 3 *bifidobacterium* only can also be
10 used for enhancing safety and efficacy of COVID-19 vaccine.

[0045] This formula can be manufactured in the pure form of powder, sachet, tablets or capsule. This synbiotic composition can also be incorporated into a food composition, for instance by dry mixing the components of the synbiotic composition successively, together or as a premix, into a food composition, following regular processing techniques. For example,
15 the food product can be powdered beverage, milk-based product, yogurt, yogurt melts, ice-cream, dry cereal mix and porridge. This synbiotic composition, and the food composition comprising such a synbiotic composition, may be used prior to COVID-19 vaccination or immediate after vaccination to improve vaccine response.

Table 2 Suggested amount per daily dose

Probiotic bacteria	Adult	Children
<i>Bifidobacterium adolescentis</i>	2.59×10 ⁵ -4.49×10 ¹¹ CFU	2.05×10 ⁵ -4.55×10 ¹¹ CFU
<i>Bifidobacterium bifidum</i>	1.26×10 ⁵ -7.35 × 10 ¹¹ CFU	1.47×10 ⁵ -3.6×10 ¹¹ CFU
<i>Bifidobacterium longum</i>	2.23×10 ⁵ – 7.02×10 ¹¹ CFU	7.55×10 ⁴ – 2.5×10 ¹¹ CFU
Prebiotics (Total weight 0.01-12g)	Adult or Children	
Xylooligosaccharide	0.01 g-6g	
Galactooligosaccharides	0.04g-9.6g	
Resistant dextrin (corn fiber)	0.01-6g	

20 CFU: colony forming unit

[0046] For probiotic bacteria, the suggested daily dosage, measured in colony-forming unit of the 3 bacteria combined, should be between 10^6 to 10^{12} CFU. For prebiotics, the suggested daily dosage when the consumed in pure form should be about 0.01g to about 12g, or about 0.1g to about 6g, or about 0.5g to about 3g, or about 1g to about 2g, or about 1.2g to about 1.5g, measured in gram(g) of the 3 prebiotics combined weight.

METHODS

Study subjects

[0047] The study protocol was approved by The Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (The Joint CUHK-NTEC CREC). Participants were fully informed of the risks of the study and signed consent forms before any study procedures. The study was conducted in accordance with good clinical practice. Eligible participants included male or female adults who were at least 18 years of age, without any known heart, lung, kidney disease, autoimmune disorders, or malignant diseases and no antibiotics were taken within 3 months. Eligible participants had received at least one dose of any type of COVID-19 vaccine (including but not limited to whole virus, protein subunit, viral vector and nucleic acid (RNA or DNA) vaccine). Adults with any known heart, lung, kidney disease, autoimmune disorders, malignant diseases, antibiotics taken within 3 months were excluded.

Blood collection and PBMC isolation

[0048] Peripheral blood samples from the subjects with COVID-19 vaccination were collected to tubes with or without EDTA. Blood in tubes without EDTA was centrifuged after 30 min of coagulation at room temperature. Serum was collected and stored in -80°C before further measurement. EDTA-anticoagulated blood samples were diluted with PBS at 1:1 ratio and transferred into Leucosep® tubes (Greiner Bio-One, Austria) filled with Ficoll-Paque Plus (sigma, German) solution. The Leucosep® tubes were centrifuged without brakes at 1400 relative centrifugal field (RCF) for 10 min at room temperature (24°C). The peripheral blood mononuclear cells (PBMCs) layer was transferred into a new tube and washed with 10 mL PBS. The cell pellets were resuspended with completed RPMI 1640 medium counted and assessed for cell viability by trypan blue exclusion test.

Stimulation of subjects' PBMCs for co-culture assay

[0049] PBMCs were seeded in 24-well tissue culture plates (Falcon, US) with the density of 6×10^5 /well. PBMCs were then cultured in RPMI1640 media with 10% FBS, 2-mercaptoethanol, L-glutamine, penicillin, streptomycin, and nonessential amino acids (Gibco, US) for 2-4 hours. After that, 1.5% PHA (v/v) (Gibco, US) and 100U/mL recombinant human IL-2 (Biolegend, US) were added into culture supernatant. 20ng/mL spike protein (SinoBiological, China) were used in SARS-CoV-2 spike protein group. Meanwhile, probiotics (*B. longum*, *B. adolescentis* and *B. bifidum*) were added into supernatant at the Multiplicity of Infection (MOI) of 20:1 for 2 hours. Probiotics culture media were collected and centrifuged at 3000g for 15 minutes to remove most bacteria. The supernatant was next filtered by 0.22 μ m filter (Millipore, Germany) before added into coculture RPMI 1640 media. PBMCs were cultured *in vitro* for 48 hours for further detection.

Cytokine detection

[0050] The cell culture medium was subjected to detection for 47 cytokines and chemokines (containing sCD40L, EGF, Eotaxin, FGF-2, FLT-3L, Fractalkine, G-CSF, GM-CSF, GRO α , IFN α 2, IFN- γ , IL-1 α , IL-1 β , IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IL-17E/IL-25, IL-17F, IL-18, IL-22, IL-27, IP-10, MCP-1, MCP-3, M-CSF, MDC, MIG, MIP-1 α , MIP-1 β , PDGF-AA, PDGF-AB/BB, TGF α , TNF α , TNF β , VEGF-A) with MILLIPLEX MAP Human Cytokine/Chemokine Kit Panel(EMD Millipore, German) following the manual. In brief, samples and standards (25 μ L) were added into the wells with 25 μ L of assay buffer, followed by adding matrix solution. After mixing, 25 μ L of magnetic beads were added to each well. The plate was incubated overnight at 4 °C with shaking. After incubation, fluid was removed, and plate was washed for 3 times. After adding detection antibodies, plate was incubated for 1 h at room temperature with shaking followed by adding streptavidin-phycoerythrin and further incubated for 30 min at room temperature. The plate was washed and added with sheath fluid. The median fluorescent intensities were determined on a Luminex®.

Antibody detection

[0051] The level of IgG and IgM antibodies were detected from serum and cell culture medium with Anti-SARS-CoV-2 IgG ELISA kit (EUROIMMUN, German) and COVID-19 N and S1 RBD protein Human IgM ELISA Kit (RayBio®, USA) according to the instructions. Absorbance was measured at 450 nm with an ELISA microplate reader.

Multicolor flow cytometry

[0052] Multicolor flow cytometry was performed to detect the composition of the B cell types in stimulated PBMCs. After coculture with probiotics or probiotic supernatant, PBMCs were collected and suspended in PBS with 1% BSA. PBMCs were then stained with fluorochrome-conjugated monoclonal antibodies of cell surface markers. CD45-PerCP/Cy 5.5, CD19-PE (Biolegend, US) were used in a flow panel. After staining, PBMCs were washed by PBS with 1% BSA and resuspended by 500 uL PBS. PBMCs samples were then filtered by 40 µm cell strainers. Flowcytometry was implemented with BD FACSCelesta™ flow cytometer (BD Biosciences, US) and the data was analyzed with BD FACSDiva software 8.0.

Statistical approach

[0053] Immune cytokines, B cells proportion, anti-SARS-CoV-2 IgG and anti-SARS-CoV-2 N and S1 RBD IgM were analysed by running non-parametric (Wilcoxon rank sum) or Student's t-test statistical tests. All statistical tests were two-sided. Statistical significance was taken as $p < 0.05$. Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) version 25.0 (SPSS, Chicago, IL).

RESULTS***Subject Characteristics***

[0054] Nine subjects who had received COVID-19 vaccine were recruited. Four of the subjects had taken an oral sachet form of the synbiotic composition which consist of 3 lyophilized *Bifidobacteria* and 3 prebiotics as described in Table 2 prior to vaccination (synbiotic group) and the other 5 subjects did not take the synbiotic composition (no synbiotic group). The characteristics of these 9 subjects are shown in **Table 3**.

Table 3 Subject characteristics

	Age	Gender	COVID19 vaccine	Date of vaccination	Synbiotic composition taken	Antibiotics taken	Comorbidity/allergy
Subject 4 WKN	83	Male	Sinovac	1 st : 2/3/2021	Yes	No	Hypertension, high cholesterol
Subject 5 WWL	43	Female	Sinovac	1 st : 3/3/2021	Yes	No	History of allergy to Iodixanol (contrast), Ceftriaxone
Subject 6 CKW	37	Female	Sinovac	1 st : 3/3/2021	No	No	No
Subject 8 TJB	57	Female	Sinovac	1 st : 3/3/2021	No	No	No
Subject 9 YWL	61	Female	Sinovac	1 st : 3/3/2021	No	No	No
Subject 10 SN	44	Female	BioNTech	1 st : 12/3/2021	Yes	No	No
Subject 11 FC	56	Male	BioNTech	1 st : 10/3/2021	Yes	No	No
Subject 12 SW	46	Male	BioNTech	1 st : 12/3/2021	No	No	No
Subject 13 PC	51	Male	BioNTech	1 st : 13/3/2021	No	No	No

The synbiotic composition reduced the level of sCD40L, IL-1 β , TNF- α , IL-17A, which are associated with adverse effects of vaccination

- 5 **[0055]** To investigate whether the synbiotic composition could enhance safety of COVID-19 vaccine, 47 cytokines and chemokines which are associated with adverse effects of vaccination were investigated. PBMCs were isolated and cultured from blood samples collected from the 9 subjects after they had COVID-19 vaccination. The 47 cytokines and chemokines in the culture medium of the PBMCs were determined by MILLIPLEX MAP
- 10 Human Cytokine/Chemokine Kit Panel. Comparing the cytokines and chemokines level in synbiotic and no synbiotic group, the inventors found that the levels of proinflammatory cytokine, sCD40L, IL-1 β , TNF- α , IL-17A, were lower in synbiotic group. The percentage drop were 80%, 28% ,14.5% and 23.1%, respectively (**Figure 1**).

Synbiotic group had increased proportion of total B cells subsets in PBMCs

- 15 **[0056]** B cell production is an indicator of vaccine efficacy. To determine whether the synbiotic composition could enhance COVID-19 vaccine efficacy, the proportion of total B cells subsets (CD45⁺CD19⁺) in PBMCs were measured by flow cytometry. The results

showed that B cells proportion in isolated PBMCs from synbiotic group is 1.47 times higher than that from no synbiotic group ($P < 0.05$, **Figure 2**).

Synbiotic group had increased production of antibodies to SARS-coV-2

[0057] To determine whether the synbiotic composition could enhance host response to COVID-19 vaccine, the concentration of IgG and IgM antibodies to SARS-CoV-2 were measured by ELISA. The results showed that concentration of IgG and IgM from synbiotic group were 1.06 and 1.2 times higher than that from no synbiotic group respectively. (**Figure 3**).

Probiotic bacteria enhanced production of IgG antibodies to SARS-CoV-2

[0058] To study the effect of the probiotic bacteria on antibody production, PBMCs isolated from one of the subjects in the no synbiotic group were co-cultured with the 3 probiotic bacteria (*B. adolescentis*, *B. bifidum*, and *B. longum*) simultaneously. PBMCs from the same subject without co-culture with probiotic bacteria was used as control. The total concentration of IgG antibodies to SARS-CoV-2 in the co-cultured medium was found to be dramatically higher than the control (**Figure 4**).

Metabolites from the 3 probiotic bacteria increased proportion of total B cells subsets in PBMCs and antibodies production

[0059] To investigate whether metabolites from the 3 probiotic bacteria had similar effect on enhancing vaccine efficacy, supernatant from the co-culture medium of the 3 probiotic bacteria was co-cultured with PBMCs isolated from subjects after COVID-19 vaccination. PBMCs from these subjects without co-culture with the supernatant was used as control. For the proportion of total B cells subsets (CD45⁺CD19⁺) in PBMCs, 5 subjects were included in the analysis (**Figure 5A**). Co-culture with metabolites increased the proportion of total B cells subsets to 1.3 times higher than that of the control group. For total concentration of IgG antibodies and IgM antibodies to SARS-CoV-2, all subjects were included in the analysis. Both IgG antibodies to SARS-CoV-2 and IgM antibodies to SARS-CoV-2-S1 RBD (**Figure 5B**) were found to have increased when co-cultured with metabolites.

[0060] All patents, patent applications, and other publications, including GenBank Accession Numbers and equivalents, cited in this application are incorporated by reference in the entirety for all purposes.

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WHAT IS CLAIMED IS:

1

1 **1.** A composition for use in enhancing vaccine efficacy and safety in a
2 subject comprising an effective amount of (1) *bifidobacteria adolescentis*, *bifidobacteria*
3 *bifidum*, and *bifidobacteria longum* or their metabolites; and (2) a physiologically acceptable
4 excipient.

1 **2.** The composition of claim 1, further comprising an effective amount of
2 xylooligosaccharides, galactooligosaccharides, and resistant dextrin (soluble corn fiber).

1 **3.** The composition of claim 1 or 2, wherein *bifidobacteria adolescentis*,
2 *bifidobacteria bifidum*, and *bifidobacteria longum* are present in the composition in the
3 weight ratio of about (0.57-3.56):1:(0.21-2.36).

1 **4.** The composition of claim 3, wherein *bifidobacteria adolescentis*,
2 *bifidobacteria bifidum*, and *bifidobacteria longum* are present in the composition in the
3 weight ratio of about (0.75-1):1:(0.75-1) for an adult subject.

1 **5.** The composition of claim 3, wherein *bifidobacteria adolescentis*,
2 *bifidobacteria bifidum*, and *bifidobacteria longum* are present in the composition in the
3 weight ratio of about (0.57-3.09):1:(0.21-1.7) for a child subject.

1 **6.** The composition of claim 2, wherein xylooligosaccharides,
2 galactooligosaccharides, and resistant dextrin (soluble corn fiber) are present in the
3 composition in the weight ratio of about (0.25-0.5):(2-4):(0.5-0.75).

1 **7.** The composition of claim 1, which is formulated for oral ingestion.

2 **8.** The composition of claim 1, which is formulated in a daily dosage
3 comprising *bifidobacteria adolescentis*, *bifidobacteria bifidum*, and *bifidobacteria longum* in
4 a combined range of about 10^6 to about 10^{12} colony-forming unit (CFU).

5 **9.** The composition of claim 2, which is formulated in a daily dosage
6 comprising xylooligosaccharides, galactooligosaccharides, and resistant dextrin (soluble corn
7 fiber) in a combined range of about 0.01 to about 6 grams, preferably about 1.2 to about 1.5
8 grams.

9 **10.** The composition of claim 1, which is in the form of a food or beverage
10 item.

11 **11.** The composition of claim 10, which is formulated in a daily dosage
12 comprising xylooligosaccharides, galactooligosaccharides, and resistant dextrin (soluble corn
13 fiber) in the amount of about 0.1 to about 12 grams.

1 **12.** A method for enhancing vaccine efficacy and safety in a subject,
2 comprising administering to the subject an effective amount of the composition of any one of
3 claims 1-11.

1 **13.** The method of claim 12, wherein the administering step comprises oral
2 ingestion of the composition.

1 **14.** The method of claim 12, wherein the administering step comprises
2 administering to the subject one composition comprising *bifidobacteria adolescentis*,
3 *bifidobacteria bifidum*, and *bifidobacteria longum*, plus xylooligosaccharides,
4 galactooligosaccharides, and resistant dextrin (soluble corn fiber).

1 **15.** The method of claim 12, wherein the administering step comprises
2 administering to the subject a first composition comprising *bifidobacteria adolescentis*,
3 *bifidobacteria bifidum*, and *bifidobacteria longum*, and administering to the subject a second
4 composition comprising xylooligosaccharides, galactooligosaccharides, and resistant dextrin
5 (soluble corn fiber).

1 **16.** The method of claim 12, wherein the subject is not diagnosed with
2 COVID-19.

1 **17.** The method of claim 12, wherein the subject has received a vaccine for
2 COVID-19 within the past 24-48 hours.

1 **18.** The method of claim 12, wherein the subject is to receive a vaccine for
2 COVID-19 within the next 24-48 hours.

1 **19.** The method of claim 12, wherein the vaccine comprises a whole virus,
2 a viral protein, a viral vector, or a nucleic acid encoding a viral protein.

- 1 **20.** A kit for enhancing vaccine efficacy and safety in a subject comprising
2 a plurality of compositions each comprising an effective amount of one or more of the
3 following: *bifidobacteria adolescentis*, *bifidobacteria bifidum*, *bifidobacteria longum*,
4 xylooligosaccharides, galactooligosaccharides. and resistant dextrin (soluble corn fiber).
- 1 **21.** The kit of claim 20, wherein the compositions are in the form of a
2 powder, liquid, paste, cream, tablet, or capsule.