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(54) Title: MATERIALS AND METHODS FOR INHIBITING A VIRAL INFECTION, INCLUDING A CORONAVIRUS INFECTION

(57) Abstract: The subject invention provides compositions and methods for inhibiting viral infections. Preferred embodiments of the invention provide pharmaceutical compositions, and the methods of using the same, comprising a strain of *Lactobacillus fermentum* bacterium, or bioactive extracts thereof.



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## MATERIALS AND METHODS FOR INHIBITING A VIRAL INFECTION, INCLUDING A CORONAVIRUS INFECTION

### CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to U.S. Provisional Patent Application Serial No. 63/035,733, filed June 6, 2020; which is incorporated herein by reference in its entirety.

### SEQUENCE LISTING

10 The Sequence Listing for this application is labeled "SeqList-05Jun20-ST25.txt," which was created on June 5, 2020, and is 3 KB. The Sequence Listing is incorporated herein by reference in its entirety.

### BACKGROUND OF THE INVENTION

Beginning in the 1960s, a variety of human-infecting coronaviruses have been identified. From the family Coronaviridae, these viruses primarily infect the upper respiratory and  
15 gastrointestinal tracts. Whilst many such infections are mild and routinely include the common cold, for example, far more pathogenic and potentially lethal strains exist, including SARS, MERS, and the 2019 outbreak strain of SARS-CoV-2, (2019-nCov or COVID-19).

SARS (Severe Acute Respiratory Syndrome), MERS (Middle East Respiratory Syndrome) and SARS-CoV-2 (SARS-related Coronavirus 2) are all highly pathogenic human coronaviruses  
20 responsible for acute and chronic diseases of the respiratory, hepatic, gastrointestinal and neurological systems. It is thought that each virus emerged from animal reservoirs and transferred to humans, thereby resulting in human epidemics, with the outbreak of SARS in 2002, MERS in 2012, and SARS-CoV-2 in late 2019.

Coronaviruses are enveloped single-stranded RNA viruses named so for their crown-like  
25 surface structure composed of spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins. The spike protein in particular is responsible for the action of entering a host cell, wherein the coronavirus is able to transcribe its RNA for intracytoplasmic replication. Indeed, coronaviruses have a unique ability to replicate and survive in the intracellular space of a macrophage, whereby multiple encoded interferon antagonists are thought to hinder the activation of type I interferon (IFN) and  
30 interferon stimulated genes (ISGs), dampening the host immune response and contributing to the resultant pathogenesis of the virus (Rose *et al.* 2010, Journal of Virology 84 (11): 5656-5669).

Upon genome replication and polyprotein formation, the viruses assemble and are released  
35 from the infected cell to further disseminate. Transmission between hosts is considered to occur primarily by contact with respiratory droplets infected with such viral particles, generated through sneezing and coughing (CDC.gov, 2020).

Coronaviruses can emerge from animal reservoirs to cause significant epidemics in humans, as exemplified by Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) in 2002-2003 and Middle East Respiratory Syndrome coronavirus (MERS-CoV), which was recognized as an emerging virus in 2012, each of which resulted in over 8000 infections and 774 deaths, and 2500 infections and 862 deaths, per respective outbreak (WHO, 2020). Declared a global emergency by the World Health Organisation (WHO), the newly discovered and rapidly disseminating SARS-CoV-2, sharing ~70% genetic similarity to the SARS-CoV, is likely to have similar epidemiological characteristics and thus presents a pressing area of healthcare concern. Crucially, there are no vaccines or antiviral drugs suitable for the prevention or treatment of human coronavirus infections at this time (Habibzadeh & Stoneman 2020, Int J Occup Environ Med 11 (2): 65-71).

A SARS-CoV-2 health emergency evidences a lack of effective virus-specific treatments or vaccines, which thus leads to a high unmet need for the protection of high-risk populations, including the elderly, health care workers and patients in acute danger of nosocomial transmission of SARS-CoV-2, or in other confined spaces, such as during quarantine settings.

Without any such viable antiviral or vaccine currently approved, there exists a particular need for a safe and effective method of treating or preventing infections or symptoms thereof caused by a coronavirus, in particular those related to SARS-CoV-2.

Biofilms are initiated when free-floating, planktonic bacteria anchor to a biologic or inert surface such as an indwelling medical device. The attached bacteria multiply and progress from a state of monolayer to a microcolony and then to a critical mass, at which point bacterial crosstalk occurs, triggering a phenomenon known as quorum sensing that leads to the biofilm phenotype. Quorum sensing can turn on biofilm-producing genes that are not expressed in planktonic bacteria. The bacteria respond collectively to express factors that are specific to the biofilm phenotype, which leads to the secretion of an exopolysaccharide (EPS) matrix, definitive of the biofilm phenotype.

The biofilm phenotype is characterized morphologically by the formation of microbial towers, which are composed of layers of embedded, live bacteria with intervening water channels. Under the right environmental conditions, free-floating bacteria are released from the biofilms, and the cycle is continued at other surfaces.

## SUMMARY OF THE INVENTION

The subject invention provides compositions and methods for inhibiting viral infections, including coronavirus infections. Preferred embodiments of the invention provide pharmaceutical compositions, and methods of using the same. In preferred embodiments, the compositions of the subject invention inhibit viral entry into cells and/or viral spread between cells via immunomodulation of the innate immune system.

Advantageously, preferred compositions and treatment methods provided herein are effective in inhibiting viral infections, including by, in some embodiments, inducing protective innate immunity. The viral inhibitory activity may also be achieved, in some embodiments, via binding of a component of the composition to the ACE2 receptor, thereby blocking entry of the virus into a cell.

5 In certain embodiments, the compositions of the subject invention can be used effectively in the treatment of acute and chronic viral infections. In specific embodiments, the subject is infected, or at risk for infection, with a coronavirus. Such coronaviruses include, for example, Severe Acute Respiratory Syndrome associated Coronavirus (SARS-CoV), Middle East Respiratory Syndrome associated Coronavirus (MERS-CoV), and the SARS-CoV-2 virus discovered in late 2019.

10 In one aspect, the subject invention provides therapeutic compositions for treating a viral infection, comprising a bacterial strain, or a bioactive extract therefrom, and a pharmaceutically acceptable excipient. In other embodiments, the subject invention provides prophylactic compositions for preventing the onset or spread of a viral infection.

In certain embodiments, the compositions comprise a strain of *Lactobacillus fermentum* 15 bacterium, or a bioactive extract thereof. In preferred embodiments, extracts of the bacteria are obtained when the bacteria are grown as biofilm. The subject invention also provides compositions comprising *L. fermentum* bacterium, or bioactive extracts thereof, in a lyophilized, freeze dried, and/or lysate form.

In some embodiments, the bacterial strain is *Lactobacillus fermentum* Qi6, also referred to 20 herein as LfQi6. In one embodiment, the subject invention provides an isolated or a biologically pure culture of LfQi6. In another embodiment, the subject invention provides a biologically pure culture of LfQi6, grown as a biofilm. Specifically taught herein are methods for inducing and identifying the biofilm phenotype. Further provided herein are methods of utilizing the biofilm phenotype, as well as extracts of the biofilm phenotype, and lysates thereof. In preferred embodiments, the pharmaceutical 25 compositions comprise bioactive extracts of LfQi6 biofilm. In one embodiment, the extract has been given the designation Qi601S.

In further embodiments, the present invention provides anti-viral proteins. In a specific embodiment, the present invention provides "Qi611S," a protein having an amino acid sequence according to **SEQ ID NO. 1**. In certain embodiments, the present invention also provides "Qi611S 30 Proteins," which include Qi611S, as well as biologically-active fragments and variants thereof.

In some embodiments, Qi611S Proteins can be produced by a cell, preferably a bacterial cell. Thus, in specific embodiments, the present invention provides methods for producing a Qi611S Protein, the methods comprise cultivating a cell having a nucleotide sequence that encodes all or a portion of **SEQ ID NO. 1**, or a variant or fragment thereof, under conditions favorable for expression

of the protein. In preferred embodiments, the nucleotide sequence is Qi611s (SEQ ID NO. 2). Optionally, the protein can be purified from the culture.

In one embodiment, the methods utilize a microorganism, e.g., *Lactobacillus fermentum* Qi6, having the Qi611s nucleotide sequence (SEQ ID NO. 2). Qi611s encodes the amino acid sequence according to SEQ ID NO. 1 (Qi611S).

In another embodiment, the cell is a microorganism that has been recombinantly altered to possess the ability to express a Qi611S Protein. In a specific embodiment, the microbe possesses all, or a portion, of the Qi611s gene. Thus, in certain embodiments, the present invention provides a recombinant cell possessing all or a portion of the DNA sequence according to SEQ ID NO. 2, and/or that is capable of expressing a protein having an amino acid sequence according to SEQ ID NO. 1, or a fragment or variant of SEQ ID NO. 1. In an exemplary embodiment, the recombinant cell is *E. coli* BL21 or *E. coli* C43.

Such transformation of cells can be accomplished using techniques well known to those skilled in the microbiological arts. In one embodiment, the nucleotide sequence can be modified to optimize expression of a Qi611S Protein.

In preferred embodiments, the present invention provides compositions comprising a Qi611S Protein and/or a cell comprising all or a portion of a DNA sequence according to SEQ ID NO. 2, and, optionally, a pharmaceutically acceptable carrier.

Pharmaceutically acceptable carriers can comprise substances used for administering the composition to a subject according to a specific route, including, for example, oral administration, inhalation, eye drops, injection (e.g., subcutaneous, intravenous, intramuscular, intraperitoneal, intrathecal, and/or subdermal), and/or topical administration (e.g., via dermal absorption). In certain embodiments, the composition can be formulated as a food item, capsule, pill, drinkable liquid, lotion, cream, emulsion, ointment, oil, gel, serum, aerosol, mist, vapor, and/or combinations thereof.

In some embodiments, the protein can be extracted and, optionally, purified from a cell culture before being formulated into the composition. In some embodiments, the composition comprises a cell capable of producing a Qi611S Protein. In one embodiment, the cell is in a lyophilized, freeze dried, and/or lysate form. In one embodiment, the composition comprises a cell culture in a biofilm state.

In another aspect, the subject invention provides a method of inhibiting a viral infection, comprising administering to a subject an effective amount of the composition, wherein the composition preferably comprises one or more bioactive extracts of the LfQi6 biofilm.

In one embodiment the subject invention provides a self-decontaminating surface wherein a protein or cell of the subject invention has been applied to the surface. The protein can be, for

example, a Qi611S protein. Preferably, the surface has antiviral properties. The surface can be , for example, cloth, textile, metal, ceramic, wood, skin, liquid, plastic or glass.

#### BRIEF DESCRIPTION OF THE FIGURES

5 Figure 1 shows a Lf Qi6 Culture procedure. Step 1: Lf Qi6 cultured in MRS agar plate; Step 2: Lf Qi6 cultured in 5 ml MRS broth for 24 hour at 37° C; Step 3: 0.1 ml of the culture is transferred into a T-150 tissue culture plate with 25 ml MRS broth; Step 4: 25 ml MRS media is changed every 48 hours, biofilm Lf Qi6 is grown on the bottom as lawn; Step 5: the culture is grown for 7 days to get a thick biofilm layer on bottom; Step 6: Biofilm layer is scraped out and suspended in fresh medium.  
10 Freezer stocks can be made with glycerol and stored in -80°.

Figure 2 illustrates a Lf Qi6 Scale-up Culture Procedure. Step 1: Biofilm phenotype Lf Qi6 is cultured in 10 ml fresh MRS medium for 24 hour at 37°; Step 2: Inoculate 10 ml culture into 25 L MRS medium with 500 g sterile glass wool; Step 3: Culture it for 72 hours in static conditions at 37°C. Mix the culture every 24 hours with a gentle shaking; Step 4: Harvest the medium and glass  
15 wool. Sonicate the glass wool to detach biofilm cells; Step 5: Centrifuge the cells to concentrate the biofilm Lf Qi6. Suspend in sterile water.

Figure 3 illustrates biofilm growth of Lf Qi6 in substrates in scale-up culture.

Figure 4 illustrates an example of Lf Qi6 downstream processing. Step 1: 50 g biofilm phenotype Lf Qi6 is suspended in 1 L of sterile water; Step 2: The suspension is gently mixed for 24  
20 hours at room temperature for passive release of bioactive substances; Step 3: the mixture is then sonicated for 30 minutes (50 KHz, 200 watt) into a uniform lysate using an OmniSonic Ruptor 400; Step 4: the sonicated lysate is frozen; Step 5: the frozen lysate is lyophilized into a fine powder.

Figure 5 illustrates SDS bands showing unique proteins in bioextract prepared from Lf Qi6 biofilm phenotype. Arrows show unique proteins in biofilm phenotype compared to planktonic  
25 phenotype.

Figure 6 illustrates size-exclusion HPLC showing unique proteins in bioextract prepared from Lf Qi6 biofilm phenotype.

Figure 7 illustrates size-exclusion HPLC showing unique proteins in bioextract prepared from Lf Qi6 biofilm phenotype.

30 Figure 8 shows a silver-stained SDS-PAGE gel used to establish the presence of Qi611S synthesized by a recombinant *E. coli* strain. The SDS-PAGE gel was loaded with samples of released protein from the recombinant *E. coli* BL21, which are identified by the bold arrow at the right of the gel. The *E. coli* strain was transformed with the Qi611s gene via the pET-15b expression vector.

Figure 9 shows a map of the pET-15b vector utilized according to embodiments of the present  
35 invention.

Figure 10 shows the pET-15b cloning/expression region of the coding strand transcribed by T7 RNA polymerase.

Figures 11-15 show control of PRCB by a composition of the subject invention at various concentrations. Essentially complete inhibition with no cytotoxicity is achieved after 60 hours. Similar results were achieved after 24 hours.

#### BRIEF DESCRIPTION OF THE SEQUENCES

**SEQ ID NO: 1** is the amino acid sequence of the protein designated as “Qi611S.”

**SEQ ID NO: 2** is *Qi611s*, a nucleotide sequence encoding the amino acid sequence of **SEQ ID NO: 1**.

**SEQ ID NO: 3** is a forward primer, “Lacto F,” useful according to the subject invention.

**SEQ ID NO: 4** is a reverse primer, “Lacto R,” useful according to the subject invention.

**SEQ ID NO: 5** is the nucleotide sequence for the cloning/expression region of the pET-15b vector.

**SEQ ID NO: 6** is an amino acid sequence for a His-tagged recombinant protein encoded by the cloning/expression region of the pET-15b vector.

#### DETAILED DESCRIPTION OF THE INVENTION

The subject invention provides compositions and methods for inhibiting a viral infection and/or the symptoms thereof. Preferred embodiments of the invention provide pharmaceutical compositions, and methods of using the same, comprising a strain of *Lactobacillus fermentum* bacterium, and/or one or more bioactive extracts thereof. The subject invention also provides compositions comprising *L. fermentum* bacterium, and/or bioactive extracts thereof, in a lyophilized, freeze dried, and/or lysate form.

Advantageously, preferred compositions and treatment methods provided herein are effective in inhibiting viral infections, including by, in some embodiments, inducing protective innate immunity. In certain embodiments, the compositions of the subject invention can be used effectively in the treatment of acute and chronic viral infections. In a specific embodiment, the subject is infected, or at risk for infection, with a coronavirus. Such coronaviruses include, for example, Severe Acute Respiratory Syndrome associated Coronavirus (SARS-CoV), Middle East Respiratory Syndrome associated Coronavirus (MERS-CoV), and the SARS-CoV-2 virus discovered in late 2019.

In one aspect, the subject invention provides a pharmaceutical composition for inhibiting a viral infection, comprising an isolated bacterial strain, and/or a bioactive extract thereof, and, optionally, one or more pharmaceutically acceptable excipients. In some embodiments, the isolated and/or biologically pure strain is *Lactobacillus fermentum* Qi6, hereafter also referred to as Lf Qi6. In preferred embodiments, the pharmaceutical compositions provided herein comprise one or more

bioactive extracts of Lf Qi6 obtained after it has been grown in the biofilm phenotype. Methods for growing biofilm are known in the art and are described in, for example, WO 2012/118535, which is incorporated herein, in its entirety, by reference, including the publications cited in that reference, such as those cited at pages 26-31.

5           The pharmaceutical compositions provided herein may also include other pharmaceutically-acceptable ingredients known to those skilled in the art, including, but not limited to, pharmaceutically-acceptable carriers, adjuvants, excipients, diluents, fillers, buffers, preservatives, anti-oxidants, lubricants, stabilisers, solubilisers, surfactants (e.g., wetting agents), masking agents, and colouring agents. The formulations may further comprise other active agents including, for  
10           example, other therapeutic or prophylactic agents.

          In another aspect, the subject invention provides a method of inhibiting, via immunomodulation or otherwise, viral infections. The method preferably comprises administering, to a subject, a therapeutically effective amount of the pharmaceutical composition, the composition preferably comprising one or more bioactive extracts of a Lf Qi6 biofilm.

15           In preferred embodiments, the compositions of the subject invention exert their virus inhibiting activity via the innate immune system. Specifically, by acting upon signaling pathways, including, for example, cytokines and receptors, involved in inflammatory and/or other immune responses, the compositions of the subject invention can inhibit the ability of the virus to enter a cell and/or spread from one cell to another. In one embodiment, the immunomodulated proceeds via the  
20           involvement of PPAR agonism. In a preferred embodiment, the innate system can thus be primed to more effectively resist future infections by the same, or related, virus. The viral inhibitory activity may also be achieved via binding of a component of the composition to the ACE2 receptor, thereby blocking entry of the virus into a cell.

## 25   **Selected Definitions**

          As used herein, reference to an “isolated” microbe refers to one that has been removed from materials with which it exists in nature. The microbe may be isolated from, for example, soil, blood, mucous, or milk such that it is removed from, and is no longer mixed or otherwise associated with, those materials to the extent that it is in nature. Such isolation can be used to impart upon the microbe  
30           markedly different characteristics, such as the production of different, or different amounts of, compounds, compared to what the microbe exhibits in its natural state.

          As used here in, a “biologically pure culture” is one that has been isolated from other biologically active materials, including any materials with which it may have been associated in nature. In a preferred embodiment, the culture has been isolated from all other living cells. In further  
35           preferred embodiments, the biologically pure culture has advantageous characteristics compared to a

culture of the same microbial species that may exist in nature. The advantageous characteristics can be, for example, enhanced production of one or more desirable growth by-products.

In certain embodiments, purified compounds are at least 60% by weight the compound of interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. For example, a purified compound is preferably one that is at least 90%, 91%, 92%, 93%, 94%, 95%, 98%, 99%, or 100% (w/w) of the desired compound by weight. Purity is measured by any appropriate standard method, for example, by column chromatography, thin layer chromatography, or high-performance liquid chromatography (HPLC) analysis.

As used herein the term “extract” refers to a composition obtained by processing a culture. The processing may involve, for example, physical and/or chemical treatment. The physical and/or chemical treatment may comprise, for example, filtering, centrifugation, sonication, pressure treatment, radiation treatment, lysing, treatment with solvents or other chemicals, and combinations of these treatments. The extract can be in the form of, for example, a supernatant such as that produced via centrifugation. The extract can also include cell mass obtained through centrifugation. The cells may be intact or not intact, viable or not viable. The extract may comprise cell membrane components and/or intracellular components. In certain embodiments, the extract is at least 80, 85, 90, or 95%, by weight, cell mass. In certain embodiments, at least 95% of the intact cells are non-viable. In certain embodiments, less than 10% of the cell mass in the extract is intact cells.

“Qi611S” refers to a protein having the amino acid sequence of **SEQ ID NO: 1**. Reference to a “Qi611S Protein,” in the singular or plural, refers to Qi611S, as well as biologically-active fragments and variants of 611S.

As used herein, “gene” refers to a segment of DNA, or a nucleotide sequence, capable of expressing a polypeptide and/or amino acid chain. In certain embodiments, the gene includes regions, such as promoter regions, preceding and/or following a coding region.

As used herein, an “isolated” or “purified” compound is substantially free of other compounds, such as cellular material, with which it is associated in nature. A purified or isolated polynucleotide (ribonucleic acid (RNA) or deoxyribonucleic acid (DNA)) is free of sequences that flank it in its naturally-occurring state. A purified or isolated polypeptide is free of, for example, other cellular material with which it would be associated in nature. “Isolated” in the context of a microbial strain means that the strain is removed from the environment in which it exists in nature. Thus, the isolated strain may exist as, for example, a biologically pure culture, or as spores (or other forms of the strain) in association with a carrier.

As provided herein, "pharmaceutically acceptable" refers to approved or approvable by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, including humans.

5 "Pharmaceutically acceptable excipient, carrier or adjuvant" refers to an excipient, carrier or adjuvant that can be administered to a subject, together with an active ingredient, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the compositions provided herein. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions of the invention is contemplated. Supplementary active ingredients can also be incorporated into the  
10 compositions.

Examples of carriers suitable for use in the pharmaceutical compositions are known in the art and such embodiments are within the purview of the invention. The pharmaceutically acceptable carriers and excipients, include, but are not limited to, aqueous vehicles, water-miscible vehicles, non-aqueous vehicles, stabilizers, solubility enhancers, isotonic agents, buffering agents, suspending and  
15 dispersing agents, wetting or emulsifying agents, complexing agents, sequestering or chelating agents, cryoprotectants, lyoprotectants, thickening agents, pH adjusting agents, and inert gases. Other suitable excipients or carriers include, but are not limited to, dextran, glucose, maltose, sorbitol, xylitol, fructose, sucrose, and trehalose.

"Planktonic" refers to a phenotype typical to microorganisms (bacteria, fungi, and/or  
20 protozoa, with associated bacteriophages and other viruses) that float freely in a liquid medium.

In some embodiments, Lf Qi6 can be grown in a biofilm phenotype. As used herein, a "biofilm" is a complex aggregate of microorganisms, such as bacteria, wherein the cells adhere to each other using a matrix usually composed of, but not limited to, polysaccharide material. The cells in biofilms have physiologically distinct properties compared to planktonic cells of the same  
25 organism, which are single cells that can float or swim in liquid or gaseous mediums, or reside on or in solid or semi-solid surfaces.

For the purpose of the present invention the abbreviation cfu shall designate a "colony forming unit" that is defined as the number of bacterial cells as revealed by microbiological counts on agar plates.

30 The term "treatment" or any grammatical variation thereof (e.g., treat, treating, etc.), as used herein, includes but is not limited to, the application or administration to a subject (or application or administration to a cell or tissue from a subject) with the purpose of delaying, slowing, stabilizing, curing, healing, alleviating, relieving, altering, remedying, less worsening, ameliorating, improving, or affecting the infection, the symptom of the infection, or the risk of (or susceptibility to) the  
35 infection. The term "treating" refers to any indication of success in the treatment or amelioration of a

pathology or condition, including any objective or subjective parameter such as abatement; remission; lessening of the rate of worsening; lessening severity of the disease; stabilization, diminishing of symptoms or making the pathology or condition more tolerable to the subject; or improving a subject's physical or mental well-being.

5           The term "prevention" or any grammatical variation thereof (e.g., prevent, preventing, etc.), as used herein, includes but is not limited to, at least the reduction of likelihood of the risk of (or susceptibility to) acquiring an infection (e.g., causing at least one of the clinical symptoms of the disease not to develop in a patient that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease). The term "prevention" may refer to avoiding,  
10       delaying, forestalling, or minimizing one or more unwanted features associated with an infection, and/or completely or almost completely preventing the development of an infection and/or its symptoms altogether. Prevention can further include, but does not require, absolute or complete prevention, meaning a condition may still develop at a later time and/or with a lesser severity than it would without preventative measures. Prevention can include reducing the severity of the onset of a  
15       disease or disorder, and/or inhibiting the progression thereof.

"Inhibition" includes direct antiviral activity against the virus, as well as directly or indirectly reducing the ability of a virus to enter a cell and/or reducing the ability of a virus to spread from one cell to another. The indirect activity may be achieved via, for example, immunomodulation.

"Immunomodulation," as contemplated by the current invention, preferably involves  
20       stimulation of innate immunity. This can involve, for example, modulation of cytokine production and/or activity as well as, in certain embodiments modulation of cellular metabolism to disfavor viral entry or replication. The modulation of cellular metabolism may be achieved by, for example, shifting mitochondrial metabolism away from sugar based metabolism to, for example, lipid based metabolism.

25       As used herein, the term "subject" refers to an animal. The animal may be, for example, a human, pig, horse, goat, cat, mouse, rat, dog, ape, fish, chimpanzee, orangutan, guinea pig, hamster, cow, sheep, bird (including chicken), as well as any other vertebrate or invertebrate.

The preferred subject, in the context of this invention, is a human of any age and/or gender. In some embodiments, the subject is suffering from a health condition, disease, or disorder, while, in  
30       some embodiments, the subject is in a state of good health (e.g., free from injury or illness) but desires enhanced health and/or functioning of a particular organ, tissue, or body system.

Administration of the pharmaceutical compositions provided herein is preferably in a "therapeutically effective amount," this being an amount sufficient to result in a biological or medical response of a cell, tissue, system, animal, or human that is being treated. The actual amount  
35       administered, and the rate and time-course of administration, will depend on the nature and severity of

the infection being treated as well as the subject. Prescription of treatment, e.g., decisions on dosage *etc.*, is within the responsibility of general practitioners and other medical doctors, and typically takes into account the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners.

5 Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 20 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, as well as all intervening decimal values between the aforementioned integers such as, for example, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, and 1.9. With respect to sub-ranges, “nested sub-ranges” that extend  
10 from either end point of the range are specifically contemplated. For example, a nested sub-range of an exemplary range of 1 to 50 may comprise 1 to 10, 1 to 20, 1 to 30, and 1 to 40 in one direction, or 50 to 40, 50 to 30, 50 to 20, and 50 to 10 in the other direction.

As used herein, “recombinant” cells are modified by the introduction of a heterologous nucleic acid, or the alteration of a native nucleic acid. Thus, for example, recombinant cells can  
15 express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under-expressed or not expressed at all.

As used herein, “reduce” refers to a negative alteration, and the term “increase” refers to a positive alteration, each of at least 1%, 5%, 10%, 25%, 50%, 75%, or 100%.

As used herein, “reference” refers to a standard or control condition.

20 The transitional term “comprising,” which is synonymous with “including,” or “containing,” is inclusive or open-ended and does not exclude additional elements or method steps not recited. By contrast, the transitional phrase “consisting of” excludes any element, step, or ingredient not specified in the claim. The transitional phrase, “consisting essentially of,” limits the scope of a claim to the specified materials or steps “and those that do not materially affect the basic and novel  
25 characteristic(s)” of the claimed invention, e.g., the ability to preclude bacterial growth. Use of the term “comprising” contemplates embodiments “consisting” and “consisting essentially” of the recited component(s).

Unless specifically stated or is obvious from context, as used herein, the term “or” is understood to be inclusive. Unless specifically stated or is obvious from context, as used herein, the  
30 terms “a,” “an,” and “the” are understood to be singular or plural.

Unless specifically stated or is obvious from context, as used herein, the term “about” is understood as within a range of normal tolerance in the art, for example, within 2 standard deviations of the mean. The term “about” can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value.

***Lactobacillus* spp. and the Qi601S Lysate**

*Lactobacillus* spp. are Gram-positive rods that are often found in fermented foods such as cheeses. Many strains are identified as probiotic because the bacteria are nonpathogenic and can inhibit pathogenic bacteria proliferation in a subject, and reduction of cholesterol.

5 In some embodiments, the bacterial strain is *Lactobacillus fermentum* Qi6, also referred to herein as LfQi6. *Lactobacillus fermentum* is a Gram-positive rod. *Lactobacillus fermentum* Qi6 (Lf Qi6) can be grown in MRS media at 37°C. In one embodiment, the subject invention provides an isolated or a biologically pure culture of LfQi6. In another embodiment, the subject invention provides a biologically pure culture of LfQi6, grown as a biofilm. Specifically taught herein are methods for inducing and identifying the biofilm phenotype. Further provided herein are methods of utilizing the biofilm phenotype, as well as extracts of the biofilm phenotype, including lysates thereof. In preferred embodiments, the pharmaceutical compositions comprise bioactive extracts of LfQi6 biofilm.

10 A culture of the *L. fermentum* microbe has been deposited with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Va. 20110-2209 USA. The deposit has been assigned accession number ATCC No. PTA-122195 by the repository and was deposited on June 10, 2015.

15 The subject culture has been deposited under conditions that assure that access to the culture will be available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 CFR 1.14 and 35 U.S.C 122. The deposit is available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

20 Further, the subject culture deposit will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., it will be stored with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposit, and in any case, for a period of at least 30 (thirty) years after the date of deposit or for the enforceable life of any patent which may issue disclosing the culture. The depositor acknowledges the duty to replace the deposit should the depository be unable to furnish a sample when requested, due to the condition of the deposit. All restrictions on the availability to the public of the subject culture deposit will be irrevocably removed upon the granting of a patent disclosing it.

25 *Lactobacillus fermentum* Qi6 (Lf Qi6) can be grown in MRS media at 37°C. Lf Qi6 is a proprietary strain. The Lf Qi6 culture can be used in the presently presented disclosure or bioactive

lysates can be isolated from the Lf Qi6 culture directly and used in antiviral methods and compositions.

Additionally, Lf Qi6 can be grown in a biofilm phenotype. The Lf Qi6 culture can be used in the presently presented disclosure or bioactive lysates can be isolated from the Lf Qi6 biofilm culture and used in the antibacterial or other barrier enhancing/creating methods and compositions.

One method to grow the culture to form a biofilm, the culture can be incubated in 5 ml of MRS broth for 24 hour at 37°C. 1 ml of the culture can then transferred into a T-150 tissue culture plate with 25 ml of MRS broth. 25 ml of MRS media can then be changed every 48 hours to allow the biofilm of Lf Qi6 to grow as lawn on the bottom of the culture plate. The culture can then be grown for 7 days to produce a thick biofilm layer. The grown biofilm layer can be subsequently scraped out and suspended in fresh medium. Freezer stocks can be made with glycerol and stored in -80°C.

A biofilm phenotype of Lf Qi6 in frozen stock can be cultured in 10 ml of fresh MRS media for 24 hour at 37°C. 10 ml of culture can be inoculated into 25 L of MRS media with 500 g of sterile glass wool. The biofilm can then be cultured for 72 hours under static conditions at 37°C. The culture can be mixed every 24 hours with a gentle shaking, after which the media and glass wool can be harvested. The biofilm cells can be subsequently detached from the glass wool via sonication. The cells can be further centrifuged to concentrate the biofilm of Lf Qi6, which was then suspended in sterile water. This scale-up yields a biofilm culture at a concentration of 2 g/L.

## **Qi611S Proteins and Polynucleotide Sequences Encoding 611S Proteins**

In preferred embodiments, the present invention provides a protein, as well as fragments and variants thereof, useful for inhibiting a viral infection. The present invention further provides nucleotide sequences that encode the protein, as well as fragments and variants thereof.

In certain specific embodiments, a protein of the present invention, referred to as “Qi611S,” has a molecular weight of about 8.0 kDA. “Qi611S proteins”, which include Qi611S and biologically-active fragments and variants thereof, can be characterized according to several parameters, including biological activities, such as, for example: antiviral activity and enhancing innate immune functions.

In certain embodiments, Qi611S Proteins can, directly or indirectly, induce expression of, and/or act as an agonist toward, one or more molecules selected from, for example, peroxisome proliferator-activated receptors (PPARs) (e.g., PPAR $\alpha$ , PPAR $\beta/\delta$ , and/or PPAR $\gamma$ ); extracellular signal-regulated kinases (ERK 1/2); and glucocorticoid receptors (GR).

Modulation of PPARs, ERK and GR can have downstream immunomodulatory effects. Thus, in certain embodiments, the composition of the subject invention can be used for inhibiting viral infections by modulating one or more biological pathways involving PPAR, ERK and/or GR.

A Qi611S Protein can further be defined by its amino acid sequence. In a specific embodiment (Qi611S), the protein has the 74 amino acid sequence shown as **SEQ ID NO: 1**.

In certain embodiments, the proteins provided herein can also be identified based on immunoreactivity with certain antibodies, as well as other methods described below.

5 In certain embodiments, Qi611S Proteins are produced by the *Lactobacillus fermentum* Qi6 bacterial strain when laboratory growth conditions are used to force the growth into a biofilm phenotype. In preferred embodiment, this bacterial strain possesses the *Qi611S* DNA sequence (**SEQ ID NO: 2**), which is capable, under biofilm phenotype conditions, of expressing a protein having **SEQ ID NO: 1**.

10 In further embodiments, a polynucleotide encoding a Qi611S Protein can be defined by, for example, the ability to hybridize with, or be amplified by, certain exemplified probes and primers (e.g., **SEQ ID NOs: 3-4**).

In certain embodiments, the present invention provides isolated polynucleotide sequences, or genes, that encode the Qi611S Proteins. Furthermore, in some embodiments, the present invention  
15 provides methods for using the polynucleotide sequences to produce recombinant hosts that express a Qi611S Protein.

In certain embodiments, the polynucleotide sequence is *Qi611S*, which is 222 base pairs and encodes Qi611S; however, in certain embodiments, different DNA sequences can encode the amino acid sequences disclosed herein because of, for example, the redundancy of the genetic code. It is well  
20 within the skill of a skilled artisan to create these alternative DNA sequences encoding the Qi611S Proteins.

As used herein, “variants” of a protein refer to sequences that have one or more amino acid substitutions, deletions, additions, or insertions. In preferred embodiments, these substitutions, deletions, additions or insertions do not materially adversely affect the therapeutic and/or cosmetic  
25 activity of Qi611S. Variants that retain one or more biological activities of Qi611S are within the scope of the present invention. Preferably the one or more biological activities are selected from antiviral activity and enhancing innate immune functions.

“Fragments” of Qi611S and its variants are also within the scope of Qi611S Proteins, so long as the fragment retains one or more biological properties of Qi611S. Preferably the one or more  
30 biological activities are selected from antiviral activity and enhancing innate immune functions. Preferably, the fragment is at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the full length Qi611S. The fragment may comprise, for example, one or more hydrophilic domains of Qi611S or variant. These domains may be directly connected with intervening amino acids removed. Hydrophilic domains can  
35 be readily identified using standard procedures known in the art.

The subject invention further contemplates fusion constructs where a Qi611S Protein is attached, directly or indirectly (e.g., via a linker), to another moiety that may be, for example, a targeting moiety (e.g., ligand, antibody, or aptamer), a carrier, a label, or an activity enhancer.

5 The subject invention further contemplates antibodies (e.g., polyclonal, monoclonal, chimeric, and humanized) to the Qi611S Proteins. These antibodies can be readily prepared by a person of ordinary skill in the art having possession of the teachings provided herein. These antibodies can be used for, for example, therapies, diagnostics, and protein purification.

10 In certain embodiments, a polynucleotide encoding a Qi611S Protein can be isolated, amplified and ligated into a vector. A “vector,” “plasmid,” or “plasmid vector” is a DNA molecule used to transfer DNA to a cell, often from one cell to another (a host cell). The vector can be replicated in the host cell; or, the vector can be a means to incorporate DNA into (or remove DNA from) a cell. A variety of means can be used to introduce a vector into a host cell. Some cells can uptake a vector without any action by one skilled in the art other than placing the vector in the cell culture. Others require chemical modification. Regardless of the means with which a cell can uptake a  
15 vector, once a host cell has the ability to do so, it is now a “competent” cell.

One example of a commercially available vector is pET-15b, in which, using restriction enzyme digestion, one skilled in the art can create a vector carrying *Qi611S*, or other polynucleotide encoding a Qi611S Protein. The pET-15b vector carries an N-terminal His•Tag® sequence followed by a thrombin site and three cloning sites (**SEQ ID NO. 6**). Unique sites are shown on the circle map depicted in **FIG. 9**. The cloning/expression region of the coding strand transcribed by T7 RNA  
20 polymerase is shown in **FIG. 10 (SEQ ID NO. 5)**.

In certain embodiments, the present invention pertains to the genetic transformation of host cells so as to provide these cells with the ability to produce a Qi611S protein. For example, a vector with *Qi611S* (or other polynucleotide encoding a Qi611S Protein) can be transformed into a host cell  
25 (e.g., a microorganism, a plant, a fungal, and/or an animal cell) allowing for the use of recombinant cells for the production of the Qi611S Protein.

In preferred embodiments, the host cell is a strain of *Escherichia coli*, e.g., *E. coli* BL21 or *E. coli* C43. Alternatively, the ability to transform cells, other than *E. coli*, into competent cells is well understood in the art, this includes cells chosen based on, e.g., their transformation ability, ability and  
30 efficiency for heterologous protein expression, stability of the protein in the host, presence of auxiliary genetic capabilities, lack of mammalian toxicity, ease of killing and fixing without damage to the protein, ease of cultivation and/or formulation, ease of handling, economics, storage stability and the like.

The present invention also provides methods of producing a Qi611S Protein by cultivating a  
35 host cell transformed with a polynucleotide of the present invention (e.g., **SEQ ID NO: 2**) under

conditions that allow for the expression of the polypeptide and, optionally, recovering the expressed polypeptide.

In certain embodiments, the host cell is transformed to express the polypeptide in enhanced amounts. For example, the DNA vector can include a strong transcriptional promoter sequence closely preceding the gene to be cloned. In certain embodiments, the strong promoter is a *trp* operon, a *lac* operon, a T7 promoter, and/or a pL promoter.

It will be recognized by those skilled in the art that DNA sequences of the subject invention may vary due to the degeneracy of the genetic code and codon usage. All DNA sequences that encode a Qi611S Protein are contemplated. Thus, all polynucleotide sequences that encode a Qi611S Protein are included in this invention, including DNA (optionally including an ATG preceding the coding region) that encodes **SEQ ID NO: 1**. The subject invention also includes polynucleotides having codons that are optimized for expression in a host cell, including any of the specific types of cells referred to herein. Various techniques for creating optimized sequences are known in the art.

Additionally, it will be recognized by those skilled in the art that allelic variations may occur in the DNA sequences that will not significantly change activity of the amino acid sequences of the peptides that the DNA sequences encode. All such variant DNA sequences are included within the scope of this invention.

The skilled artisan will understand that the exemplified sequences can be used to identify, produce, and use additional nucleotide sequences that encode Qi611S Proteins. Variant DNA sequences having at least 90%, or at least 95% identity to a recited DNA sequence and encoding a Qi611S Protein are included in the subject invention. Other numeric ranges for variant polynucleotides and amino acid sequences are provided below (e.g., 50-99%). Following the teachings herein and using knowledge and techniques well known in the art, the skilled worker will be able to make a large number of operative embodiments having variant DNA sequences without the use of undue experimentation. Specifically contemplated are homologs from other strains or species.

The fragments and the mutational, insertional, and deletional variants of the polynucleotide and amino acid sequences of the invention can be used in the same manner as the exemplified sequences so long as the fragments and variants have substantial sequence similarity with the original sequence. As used herein, substantial sequence similarity refers to the extent of nucleotide or amino acid sequence similarity that is sufficient to enable the variant or fragment sequence to function in the capacity as the original sequence. Preferably, this similarity is greater than 50%; more preferably, this similarity is greater than 75%; and most preferably, this similarity is greater than 90%. The degree of similarity needed for the variant to function in its intended capacity will depend upon the intended use of the sequence. It is well within the skill of a person trained in this art to make mutational, insertional, and deletional mutations that are designed to improve the function of the sequence or

otherwise provide a methodological advantage. The identity and/or similarity can also be 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% as compared to a sequence exemplified herein.

5 The amino acid identity/similarity and/or homology will typically be highest in critical regions of the protein that account for biological activity and/or are involved in the determination of three-dimensional configuration that ultimately is responsible for the biological activity. In this regard, certain amino acid substitutions are acceptable and can be expected if these substitutions are in regions that are not critical to activity or are conservative amino acid substitutions that do not affect  
10 the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions, whereby an amino acid of one class is replaced with another amino acid of the same type, fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. The following (Table 1) is a list of examples of amino acids belonging to each class.

15

Table 1. Classification of amino acids based on physical properties.

| Class of Amino Acid | Examples of Amino Acids                |
|---------------------|--|
| Nonpolar            | Ala, Val, Leu, Ile, Pro, Met, Phe, Trp |
| Uncharged Polar     | Gly, Ser, Thr, Cys, Tyr, Asn, Gln      |
| Acidic              | Asp, Glu                               |
| Basic               | Lys, Arg, His                          |

In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not significantly detract from the biological activity of the protein.

20

### Viruses and Diseases Treated

In one embodiment, the compositions and methods of the subject invention can be used to inhibit a viral infection caused by, for example, an RNA virus including retroviruses (e.g., lentiviruses such as HIV) and coronavirus (e.g., the virus that causes COVID-19). Examples of RNA viruses  
25 include, but are not limited to, Orthomyxoviruses, Adenovirus, Hepatitis C Virus (HCV), Zika, the virus that causes COVID-19, Dengue, Ebola, Influenza A/B/C virus, polio measles, primate foamy virus, HIV, SARS-CoV (Severe Acute Respiratory Syndrome-Corona Virus), CoV MERS (Middle East Respiratory Syndrome virus), HCoV-NL63, HCoV-OC43, HCoV-229E, HCoV-HKU1, adult T-cell leukemia virus (ATLV), Human T-cell lymphotropic virus type 1 (HTLV-1), and type II (HTLV-  
30 2). Furthermore, inhibition of mutated and/or truncated viruses derived from the above viruses are

encompassed by the present invention.

SARS-CoV, MERS-CoV and SARS-CoV-2 are all highly pathogenic human coronaviruses responsible for acute and chronic diseases of, for example, the respiratory, hepatic, gastrointestinal and neurological systems. It is envisaged that the administration of a composition of the subject invention may be used for the treatment and/or prevention of any infection caused by any coronavirus.

In a particular embodiment, the coronavirus may be SARS-CoV. In another embodiment, the coronavirus may be MERS-CoV. In a preferred embodiment, the coronavirus may be SARS-CoV-2.

Other viruses include, for example, avian leukemia virus, avian sarcoma virus, avian reticuloendotheliosis virus, murine mammary cancer virus, murine leukemia virus, murine sarcoma virus, guinea pig type C virus, hamster type C virus, rat leukemia virus, feline leukemia virus, feline sarcoma virus, feline type C virus, ovine leukemia virus, bovine leukemia virus, swine type C virus, simian leukemia virus, Mason-Pfizer virus, simian sarcoma virus, simian T-lymphotropic virus, baboon type C virus, visna virus, EIAV, spuma virus, ovine progressive pneumonia virus, ovine maedi virus, simian T-lymphotropic virus type III (STLV-III), equine infectious anemia virus, Bovine immunodeficiency virus (BIV), the Feline immunodeficiency virus (FIV), the Simian immunodeficiency virus (SIV), feline Coronavirus (FCoV), and Mouse Hepatitis Virus (MHV-LUC).

In one embodiment, the subject invention provides methods for preventing and/or treating a disease, or symptoms of a disease, caused by a virus, e.g., a retrovirus or coronavirus. The method comprises administering the composition of the subject invention to a subject in need of such prevention and/or treatment.

In one embodiment, the disease may be, for example, Zika, Ebola, Hepatitis C, Influenza, COVID-19, MERS, SARS, AIDS, adult T-cell lymphoma (ATL), Dengue fever, and progressive general lymphadenosis (PGL). Preferably, the disease is COVID-19.

## **25 Pharmaceutical Compositions of the Subject Invention**

A composition of the subject invention may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. The pharmaceutical compositions provided herein may be dissolved in, suspended in, or admixed with one or more other pharmaceutically acceptable ingredients. The compositions may also be presented in a liposome or other microparticle.

The pharmaceutical compositions provided herein may contain a single (unit) dose of bacteria, or lysate, or extract thereof. Suitable doses of bacteria (intact, lysed or extracted) may be in the range 10<sup>4</sup> to 10<sup>12</sup> cfu, e.g., one of 10<sup>4</sup> to 10<sup>10</sup>, 10<sup>4</sup> to 10<sup>8</sup>, 10<sup>6</sup> to 10<sup>12</sup>, 10<sup>6</sup> to 10<sup>10</sup>, or 10<sup>6</sup> to 10<sup>8</sup> cfu. In some embodiments, doses may be administered once or twice daily. In some

0.01 %, about 0.05%, about 0.1 %, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1.0%, about 1.5%, about 2.0%, about 3.0%, about 4.0%, about 5.0%, about 6.0%, about 7.0%, about 8.0%, about 9.0%, about 10.0%, about 11.0%, about 12.0%, about 13.0%, about 14.0%, about 15.0%, about 16.0%, about 17.0%, about 18.0%, about 19.0%, about 20.0%, about 25.0%, about 30.0%, about 35.0%, about 40.0%, about 45.0%, about 50.0% by weight of the Lf Qi6 extracts. In some embodiments, the compositions may comprise, one of at least about 0.01 % to about 30%, about 0.01 % to about 20%, about 0.01 % to about 5%, about 0.1 % to about 30%, about 0.1 % to about 20%, about 0.1 % to about 15%, about 0.1 % to about 10%, about 0.1 % to about 5%, about 0.2% to about 5%, about 0.3% to about 5%, about 0.4% to about 5%, about 0.5% to about 5%, about 1 % to 10 about 5%, by weight of the Lf Qi6 extracts.

The compositions are preferably formulated into suitable pharmaceutical preparations such as aerosols, inhalants, tablets, capsules, or elixirs for oral administration or in sterile solutions or suspensions for parenteral administration.

In one embodiment, the composition according to the invention, together with a conventional adjuvant, carrier, or diluent, may thus be placed into the form of solids including pills, troches, tablets, cachets, lozenges, filled capsules, powder and pellet forms, and liquids such as aqueous or non-aqueous solutions, suspensions, emulsions, elixirs, and capsules filled with the same. The composition may further comprise conventional ingredients in conventional proportions, with or without additional active compounds.

The tablets, troches, pills, capsules, and the like may also contain one or more of the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid, and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose, or aspartame, or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. Various other materials may be present as coatings or for otherwise modifying the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac, or sugar, and the like.

The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the biopolymer-based hydrogel nanoparticles and microparticles. Preferably, the ultimate dosage form should be sterile, fluid, and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof.

Pharmaceutical compositions for topical administration can be formulated as ointments,

creams, lotions, gels, or as a transdermal patch. Such transdermal patches can contain penetration enhancers such as linalool, carvacrol, thymol, citral, menthol, t-anethole, and the like. Ointments and creams can, for example, include an aqueous or oily base with the addition of suitable thickening agents, gelling agents, colorants, and the like. Lotions and creams can include an aqueous or oily base  
5 and typically also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, coloring agents, and the like. Gels preferably include an aqueous carrier base and include a gelling agent such as cross-linked polyacrylic acid polymer, a derivatized polysaccharide (*e.g.*, carboxymethyl cellulose), and the like.

Pharmaceutical compositions suitable for intra-nasal administration are also encompassed by  
10 the present invention. Such intra-nasal compositions comprise the active composition in a vehicle and suitable administration device to deliver a liquid spray, dispersible powder, or drops. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents, or suspending agents. Liquid sprays are conveniently delivered from a pressurized pack, an insufflator, a nebulizer, or other convenient means of delivering an aerosol. The  
15 active composition may be combined with an inert powdered carrier and inhaled by the subject or insufflated.

Pharmaceutical compositions for administration by inhalation or insufflation can be provided in the form of a dry powder composition, and a suitable powder base such as lactose or starch. Such powder composition can be provided in unit dosage form, for example, in capsules, cartridges, gelatin  
20 packs, or blister packs, from which the powder can be administered with the aid of an inhalator or insufflator. In a preferred embodiment, the compositions are administered in an inhalant form.

In one embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for local administration to humans. Typically, compositions for local administration are solutions in a sterile isotonic aqueous buffer. Where necessary, the  
25 composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is administered by injection, an ampoule of sterile water for injection or saline  
30 can be provided so that the ingredients may be mixed prior to administration.

In some embodiments the composition may be administered in an intradermal injection via a microneedle device comprising a plurality of needles. In another embodiment, the plurality of microneedles may be deployed in a line, square, circle, grid, or array. In other embodiments, the microneedle device may include between 2 and 2000 microneedles per square centimetre, such as  
35 between 4 and 1500 microneedles per square centimetre, of between 10 and 1000 microneedles per

square centimetre. In a further embodiment, the microneedles may be between 2 and 2000 microns in length, such as between 20 and 1000 microns, or between 50 and 500 microns, or between 100 and 400 microns.

In some embodiments, the microneedles are solid. In other embodiments, the microneedles are hollow. In a further embodiment, the microneedles may be configured to deliver the composition intradermally, optionally wherein said immunomodulator is delivered to the lymphatic vessels. In yet a further embodiment, the composition may be coated onto or embedded within at least a portion of the microneedles, optionally wherein the microneedles are implanted into or removable from the skin. Preferably, said coating or microneedle would be dissolvable upon contact with the skin.

## 10 **Methods of Administration**

In certain embodiments, the compositions of the subject invention may be administered by inhalation, orally, intra-nasally, topically, intramuscularly, subcutaneously, intrathecally, intravenously or intraperitoneally by infusion or injection.

In some embodiments of the invention, the composition may be administered via a single-use pre-filled syringe. In other embodiments, the composition may be administered via an automated, multi-use, or disposable cartridge jet injector, such as the 'Tropis ID' (WHO approved for polio) and 'Stratis' device, which is FDA approved for flu (both ex Pharmajet).

In some embodiments of the present invention, the composition may be administered for the treatment or prevention of infections caused by a virus in combination with one or more other antiviral therapies. Such antiviral therapies may include administration of oseltamivir phosphate (Tamiflu®), zanamivir (Relenza®), peramivir (Rapivab®), baloxavir marboxil (Xofluza®), or lopinavir/ritonavir (Aluvia®). Such antiviral therapies may be administered simultaneously, separately or sequentially with the subject composition. In a further embodiment, the antiviral therapy is administered via the same or different route of administration as the subject composition.

The particular mode of administration and the dosage regimen will be selected by the attending clinician, taking into account the particulars of the case (*e.g.*, the subject, the disease, the disease state involved, and whether the treatment is prophylactic). Treatment may involve daily or multi-daily doses of compound(s) over a period of a few days to months, or even years.

In general, however, a suitable dose will be in the range of from about 0.001 to about 100 mg/kg of body weight per day, preferably from about 0.01 to about 100 mg/kg of body weight per day, more preferably, from about 0.1 to about 50 mg/kg of body weight per day, or even more preferred, in a range of from about 1 to about 10 mg/kg of body weight per day. For example, a suitable dose may be about 1 mg/kg, 10 mg/kg, or 50 mg/kg of body weight per day.

The compositions can be conveniently administered in unit dosage form, containing for example, about 0.05 to about 10000 mg, about 0.5 to about 10000 mg, about 5 to about 1000 mg, or

about 50 to about 500 mg of the active ingredient per unit dosage form.

The compositions may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as one dose per day or as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, *e.g.*, into a number of discrete loosely spaced administrations.

The composition may be used in prophylaxis of populations in general, but also those at high risk of virus infection, including health care workers and persons who have been in close contact to infected patients, as well as the elderly. Such prophylaxis may be achieved by changing the immune surveillance status and disease trajectory. It is also envisaged that an immunomodulating composition of the present invention may be used as a co-treatment of already infected patients.

Optionally, the pharmaceutical compositions of the present invention can include one or more other therapeutic agents, *e.g.*, as a combination therapy. The additional therapeutic agent(s) will be included in the compositions within a therapeutically useful and effective concentration range, as determined by routine methods that are well known in the medical and pharmaceutical arts. The concentration of any particular additional therapeutic agent may be in the same range as is typical for use of that agent as a monotherapy, or the concentration may be lower than a typical monotherapy concentration if there is a synergy.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

## **Materials and methods**

### ***Culture of Lf Qi6 biofilm***

Figure 1 shows procedures for seeding the Lf Qi6 culture. After being isolated and identified, Lf Qi6 was cultured in MRS agar plate. The culture was then incubated in 5 ml of MRS broth for 24 hour at 37° C. 1 ml of the culture was transferred into a T-150 tissue culture plate with 25 ml of MRS broth. 25 ml of MRS media was changed every 48 hours to allow the biofilm of Lf Qi6 to grow as lawn on the bottom of the culture plate. The culture was then grown for 7 days to produce a thick biofilm layer. The grown biofilm layer was subsequently scraped out and suspended in fresh medium. Freezer stacks were made with glycerol and stored in -80°.

A scaled-up production of Lf Qi6 biofilm is illustrated in Figure 2. A biofilm phenotype of Lf Qi6 in frozen stock was cultured in 10 ml of fresh MRS media for 24 hour at 37°. 10 ml of culture was inoculated into 25 L of MRS media with 500 g sterile glass wool. The biofilm was then cultured for 72 hours under static conditions at 37°C. The culture was mixed every 24 hours with a gentle shaking, after which the media and glass wool were harvested. The biofilm cells were subsequently

detached from the glass wool via sonication. The cells were further centrifuged to concentrate the biofilm of Lf Qi6, which was then suspended in sterile water. This scale-up yields a biofilm culture at a concentration of 50 g/25 L. The Lf Qi6 biofilm growth is further illustrated in Figure 3, wherein the biofilm was cultured on substrates in a scaled-up culture as described herein.

5

#### ***Lf Qi6 biofilm downstream processing***

The downstream processing of Lf Qi6 biofilm is shown in Figure 4. 50 g of biofilm phenotype of Lf Qi6 was suspended in 1 L of sterile water. The suspension was gently mixed for 24 hours at room temperature to allow the passive release of multiple bioactives. The mixture was then sonicated for 30 minutes (50 KHz, 200 watt) into uniform lysate using an OmniSonic Ruptor 400. The sonicated lysate was then frozen and lyophilized into a fine powder.

10

#### ***Preparation of Lf Qi6 from probiotic bacteria***

*L. fermentum Qi6* was grown in MRS media using proprietary culture methods. Bacteria were then subcultured into 500ml MRS medium for an additional period, again using proprietary culture methods. Bacteria were sonicated (Reliance Sonic 550, STERIS Corporation, Mentor, OH, USA), centrifuged at 10,000g, cell pellets dispersed in sterile water, harvested cells lysed (Sonic Ruptor 400, OMNI International, Kennesaw, GA, USA) and centrifuged again at 10,000 g, and soluble fraction centrifuged (50kDa Amicon Ultra membrane filter, EMD Millipore Corporation, Darmstadt, Germany, Cat#UFC905008). The resulting fraction was distributed into 0.5ml aliquots, flash frozen in liquid nitrogen and stored at -80°C.

15

20

Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting.

#### **EXAMPLE 1 – *Lf Qi6 biofilm phenotype differs from the planktonic phenotype***

Product extraction and protein estimation demonstrate different protein levels between the biofilm and planktonic phenotypes, as seen in Table 2 below. SDS data in Figure 5 further corroborate such differences, as shown by extra bands expressed in the Lf Qi6 biofilm phenotype that are not present in the planktonic phenotype, indicative of unique proteins in the bioextract of the biofilm. Size-exclusion HPLC data in Figures 6 and 7 illustrate differences in molecular weight between proteins in bioextract prepared from Lf Qi6 biofilm and planktonic phenotype.

30

---

Table 2: Product extraction and protein estimation from biofilm and planktonic shows different protein levels.

---

|               | <i>Sample No.</i> | <i>Description</i>  | <i>Extract Yield (g/L)</i> | <i>Protein Yield (g/L)</i> | <i>Protein % In Extract</i> |
|---------------|-------------------|---------------------|----------------------------|----------------------------|-----------------------------|
| Pellet        | 1                 | Planktonic          | 0.4                        | 0.2                        | 52.2                        |
|               | 2                 | Biofilm-unextracted | 0.7                        | 0.4                        | 61.3                        |
|               | 3                 | Biofilm-extracted   | 0.6                        | 0.2                        | 37.3                        |
| Cell Lysate   | 1                 | Planktonic          | 0.09                       | 0.03                       | 30.8                        |
|               | 3                 | Biofilm-extracted   | 0.10                       | 0.04                       | 34.7                        |
|               | 4                 | Biofilm-unextracted | 0.2                        | 0.06                       | 31.6                        |
| Biosurfactant | 1                 | Planktonic          | 0.12                       | 0.05                       | 42.2                        |
|               | 3                 | Biofilm-extracted   | 0.16                       | 0.06                       | 36.7                        |

#### EXAMPLE 2 – ISOLATION AND TRANSFORMATION OF *Qi611S* GENE INTO *E. coli* BL21

A gene encoding Qi611S, *Qi611S*, can be isolated from *Lactobacillus fermentum* Qi6 and transformed into *E. coli* BL21. As shown in **FIG. 8**, *E. coli* BL21 is capable of synthesizing a Qi611S protein.

Primers that were used to amplify *Qi611S* from LF Qi6 can be used to confirm the presence of *Qi611S* in the *E. coli* cell. The primers used for cloning of *Qi611S* into the pET-15b vector are listed in **Table 3** (see also **FIGS. 9-10**). These specially-designed primers were obtained from Integrated DNA Technologies (IDT) (Coralville, Iowa).

**Table 3. Primers used in the cloning of *Qi611S* for transformation into *E. coli* BL21.**

| <b>Primer Name</b> | <b>Primer Sequence (5'-3')</b>           |
|--------------------|--|
| Lacto F            | CATATGGATAACCGGATTTTCTTCA (SEQ ID NO: 3) |
| Lacto R            | GGATCCTTAGAGGTTTTTCTTAATC (SEQ ID NO: 4) |

DNA was isolated and amplified from the Lf Qi6 chromosome that encodes Qi611S using the primers featured in **Table 3**. The primers enabled the use of two restriction enzymes, NdeI and BamHI, for a restriction enzyme digest of the amplified *Qi611S* gene and the multiple cloning site of the pET-15b vector.

Once the amplified DNA and the vector are digested using the restriction enzymes, the *Qi611S* gene is ligated into the vector. The vector can then be transformed into *E. coli* BL21.

The transformed *E. coli* culture is grown overnight at 37°C and individual colonies are tested for the presence of *Qi611S* using PCR. Once positive clones are identified, the bacteria can then be grown to identify if *Qi611S* is successfully encoding synthesis of Qi611S.

5 The Qi611S protein can be detected by silver staining on SDS-PAGE gel. The SDS-PAGE gel is loaded with released protein samples from *E. coli* BL21 that is expressing Qi611S. The pET-15b vector encodes a polyhistidine-tag, enabling the identification of a synthesized protein without having an antibody specific to the Qi611S. Additionally, the pET-15b encodes the thrombin protease cleavage site (Leu-Val-Pro-Arg-Gly-Ser) to enable removal of the polyhistidine-tag.

### 10 EXAMPLE 3 – ANTIVIRAL ACTIVITY AGAINST PORCINE RESPIRATORY CORONAVIRUS

Porcine respiratory coronavirus (PRCV) is a single-stranded, negative-sense, RNA virus in the family of *Coronaviridae*. It was first identified in Belgium in 1984. PRCV is a deletion mutant of the enteric coronavirus transmissible gastroenteritis virus (TGEV) and is also closely related to feline enteric coronavirus and canine coronavirus.

15 PRCV is spread via aerosol and direct contact between pigs. This usually occurs post-weaning when maternally derived antibody-mediated protection begins to decline. Transmission may also occur in growers/finishers when PRCV-naïve pigs are introduced.

An extract from the biofilm phenotype of LfQi6, designated Qi601S, was tested for activity against PRCV. The tests were conducted in porcine Sertoli (ST) cells. As shown in Figures 11-15, 60  
20 hours following co-incubation of a high inoculum dose of the PRCV in porcine Sertoli cells (ST) shows about 100% protection using 5% Qi601S, with no toxicity in cells unchallenged with the PRCV.

25 It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

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## CLAIMS

What is claimed is:

1. A method of inhibiting a viral infection in a subject's body, wherein the method comprises administering to the subject an effective amount of:
  - A) a protein that comprises an amino acid sequence selected from:
    - i) SEQ ID NO: 1;
    - ii) a variant of SEQ ID NO: 1; and
    - iii) a fragment of i) or ii)wherein said variant or fragment has virus-inhibiting activity; or
  - a composition comprising an isolated bioactive *Lactobacillus fermentum* bacterial strain grown as biofilm, and/or comprising a viral inhibiting extract of said biofilm; and/or
  - B) a composition comprising an isolated bioactive *Lactobacillus fermentum* bacterial strain grown as biofilm, and/or comprising a viral inhibiting extract of said biofilm.
2. The method of claim 1, wherein the protein comprises SEQ ID NO: 1, or is a variant of SEQ ID NO: 1 having at least 75% sequence identity to SEQ ID NO: 1, or which is a fragment of SEQ ID NO: 1 or a fragment of said variant, wherein said fragment has a length of at least 20% the length of SEQ ID NO: 1.
3. The method of claim 1, wherein the protein comprises SEQ ID NO: 1.
4. The method of claim 1, wherein the protein has SEQ ID NO: 1.
5. The method of claim 1, wherein the protein comprises a fragment of at least 70% of SEQ ID NO: 1.
6. The method, according to claim 1, wherein the bacterial strain is *Lactobacillus fermentum* Qi6 having Accession No. PTA-122195.
7. The method of claim 1, wherein the virus is an RNA virus.
8. The method of claim 7, wherein the virus is coronavirus.
9. The method of claim 7, wherein the coronavirus is SARS-CoV-2.

10. The method of claim 7, wherein the coronavirus is SARS-CoV.
11. The method of claim 7, wherein the coronavirus is MERS-CoV.
12. The method of claim 1, wherein the composition is administered via a parental, oral, sublingual, nasal or pulmonary route.
13. The method of claim 12, wherein the parental route is selected from subcutaneous, intradermal, subdermal, intraperitoneal and intravenous injection.
14. The method of claim 13, wherein the parental route is an intradermal injection.
15. The method of claim 1, wherein the composition is administered in combination with one or more other antiviral therapies.
16. The method of claim 1, wherein the ability of the virus to enter a cell, or spread to a new cell is inhibited.
17. The method of claim 1, wherein viral inhibitory activity is achieved via the innate immune system.
18. The method of claim 1, wherein the subject is a human.
19. The method of claim 1, further comprising the administration to the subject of an additional antiviral agent.
20. The method of claim 6, comprising administration to the subject of an extract of the said bacterial strain.
21. The method of claim 1, wherein the composition is administered via a ventilator.
22. A self-decontaminating surface having antiviral properties wherein said surface comprises:
  - A) a protein that comprises an amino acid sequence selected from:
    - i) SEQ ID NO: 1;

- ii) a variant of SEQ ID NO: 1; and
- iii) a fragment of i) or ii)

wherein said variant or fragment has virus-inhibiting activity; or

a composition comprising an isolated bioactive *Lactobacillus fermentum* bacterial strain grown as biofilm, and/or comprising a viral inhibiting extract of said biofilm; and/or

B) a composition comprising an isolated bioactive *Lactobacillus fermentum* bacterial strain grown as biofilm, and/or comprising a viral inhibiting extract of said biofilm.

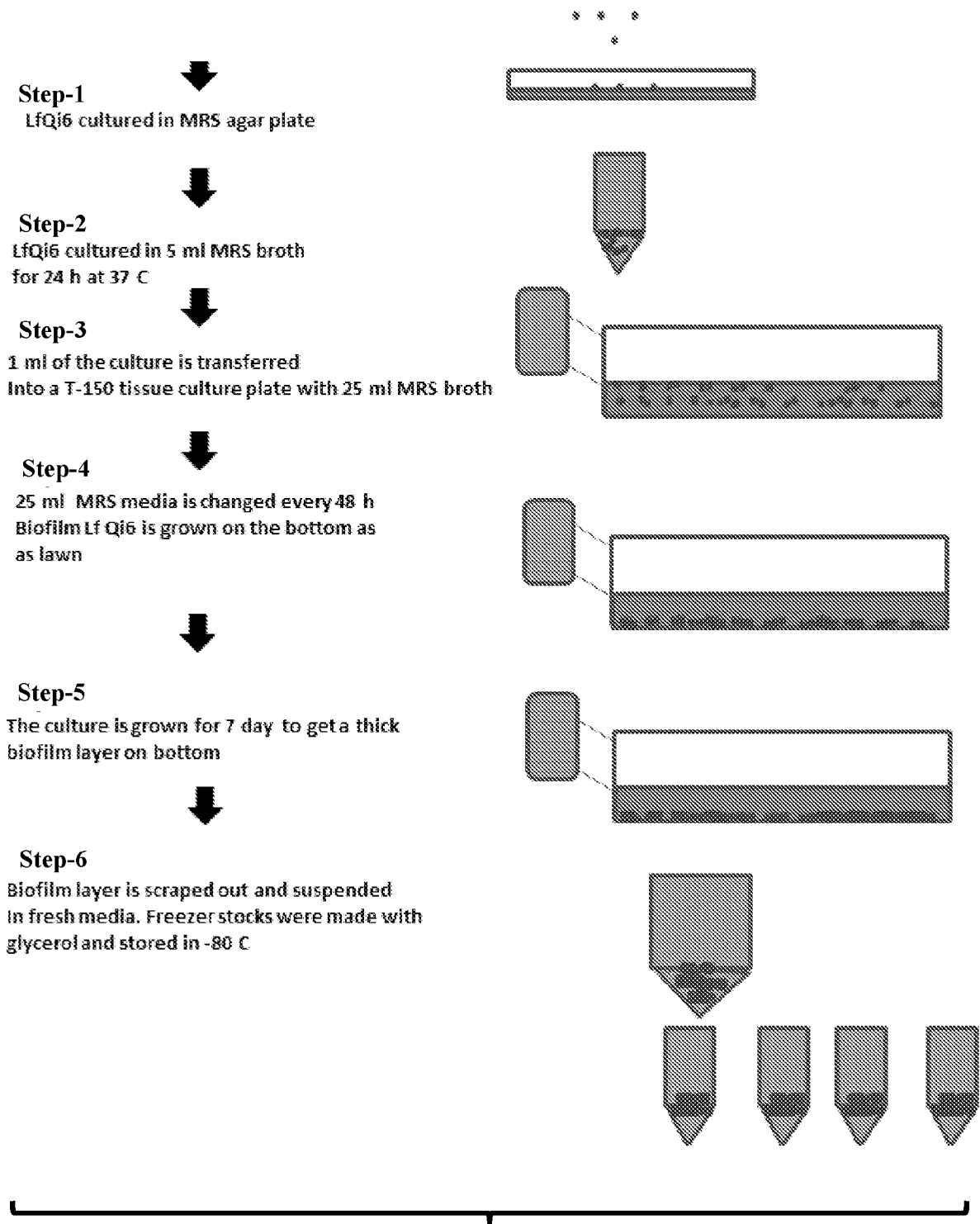


FIG. 1

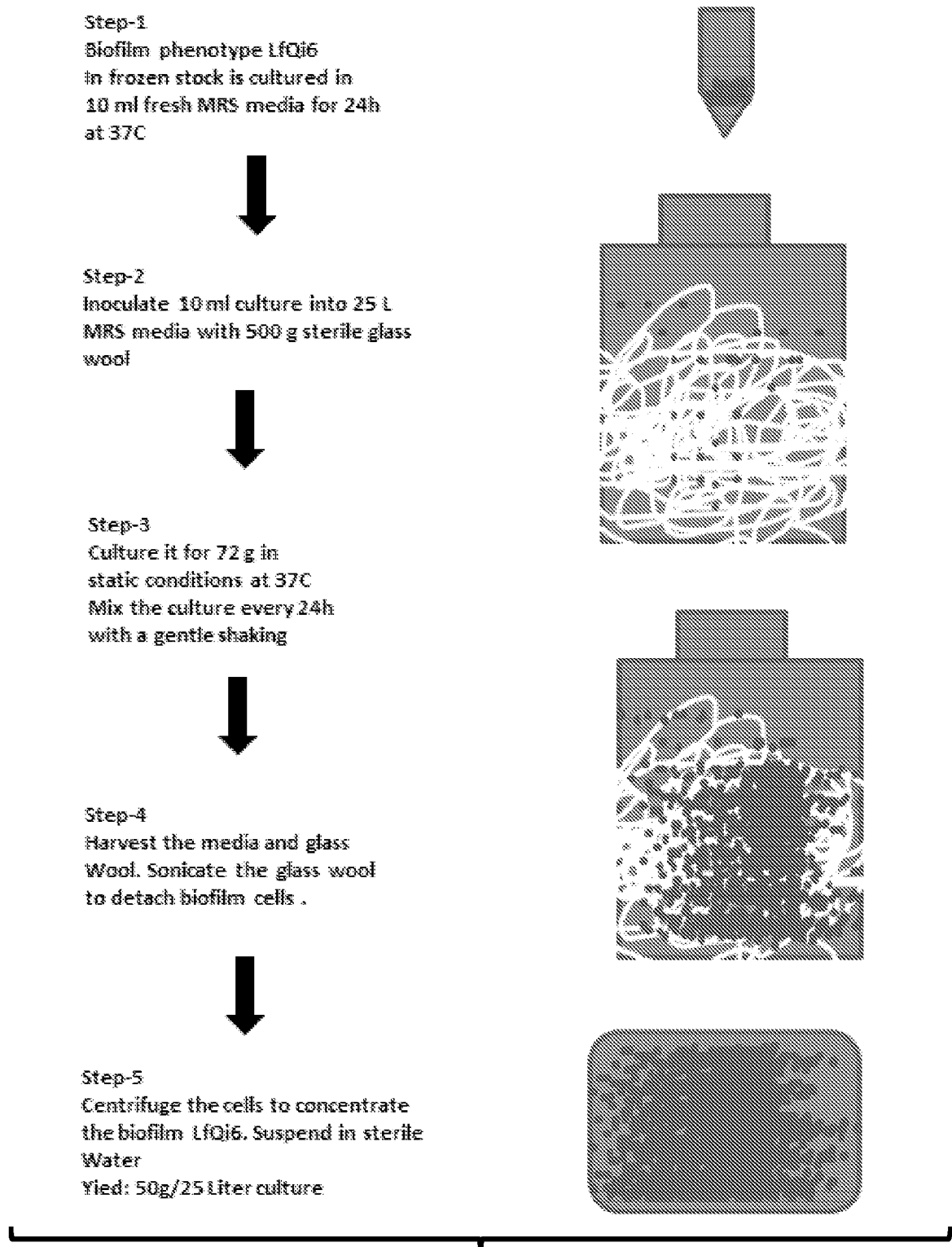


FIG. 2

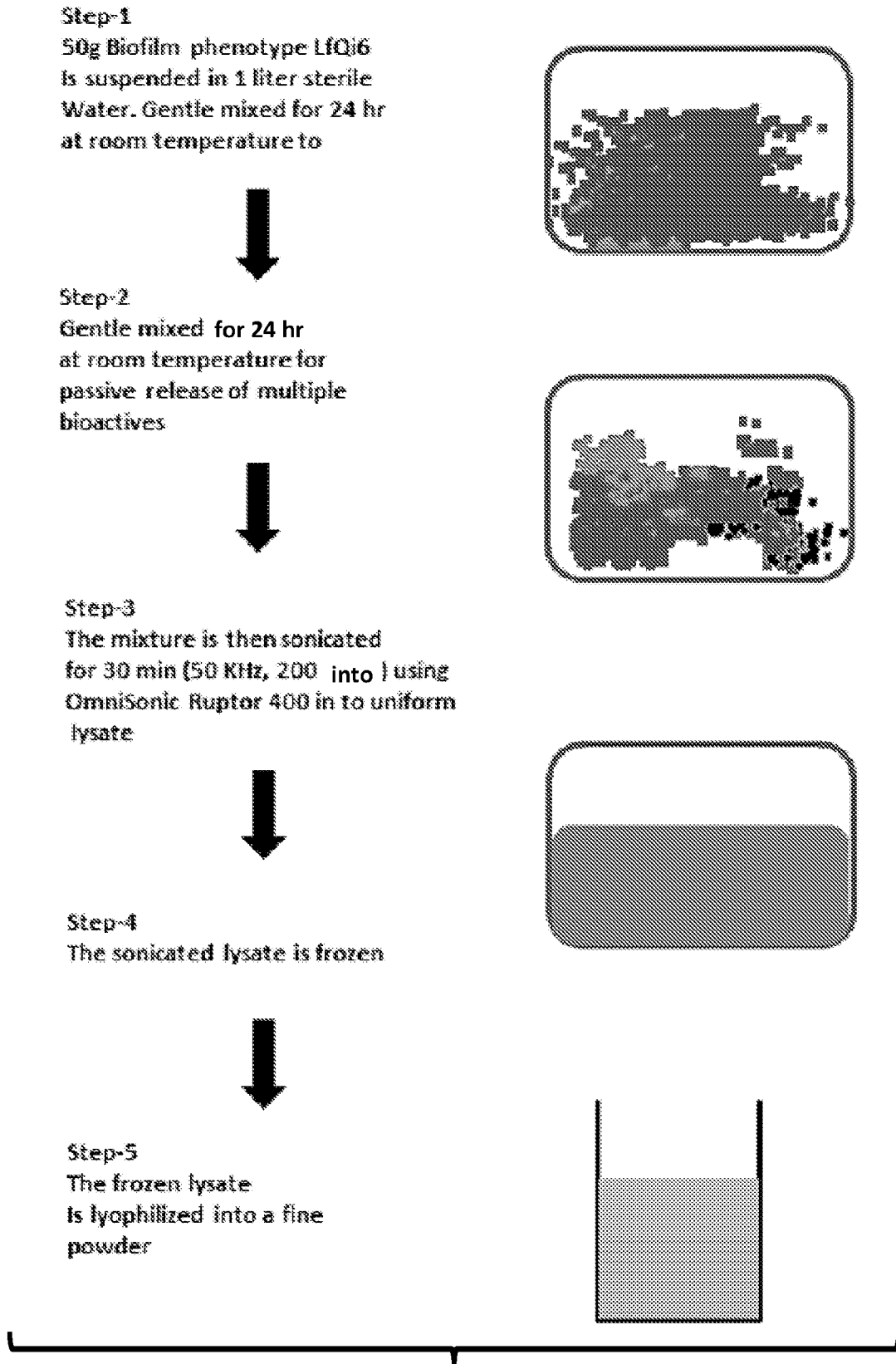


FIG. 4

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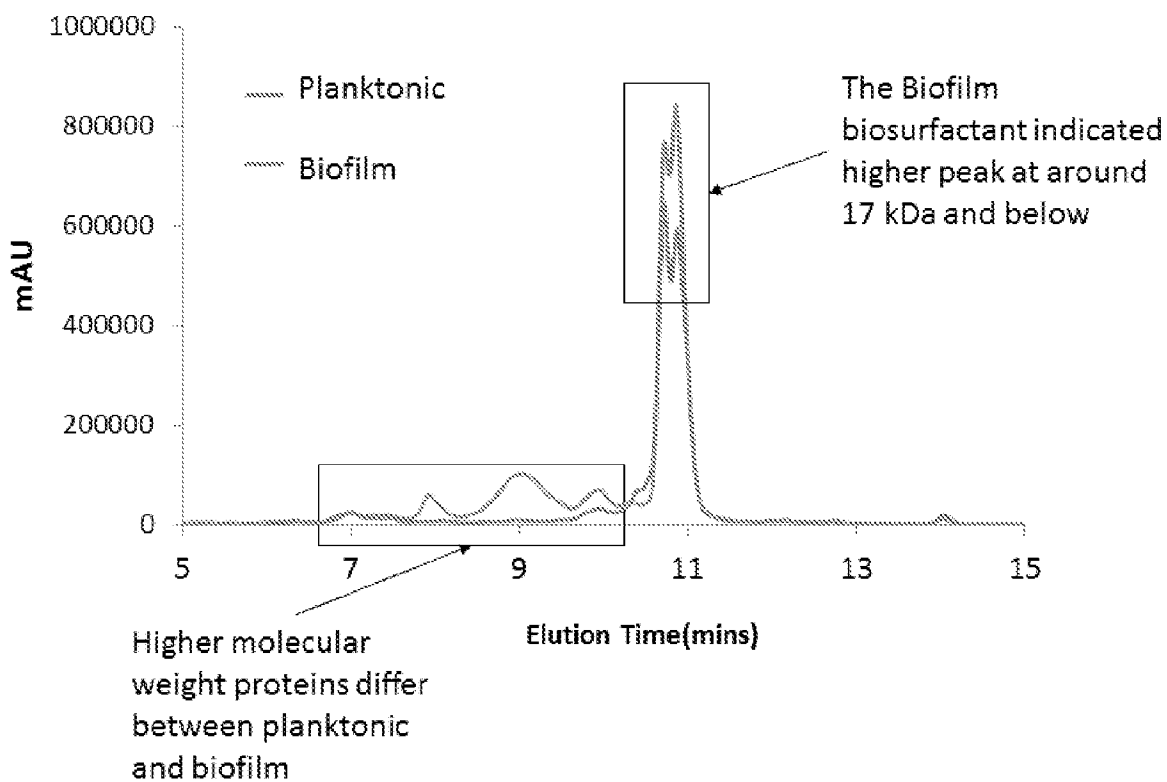


FIG. 6

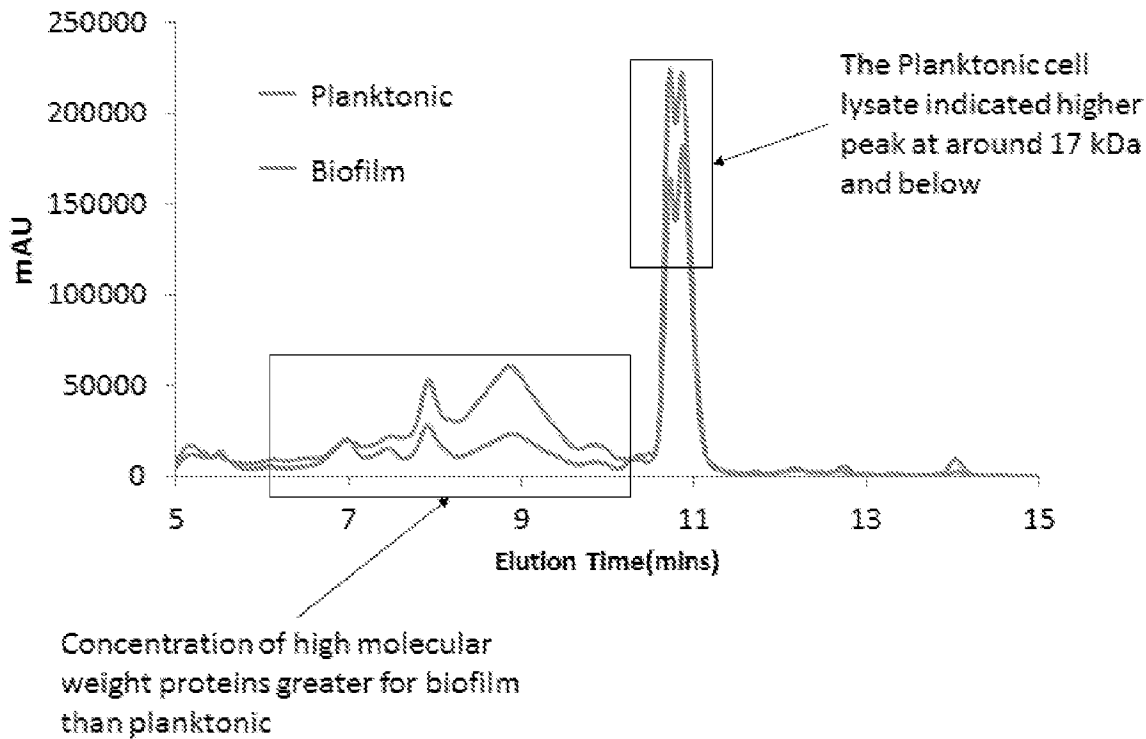


FIG. 7

7/12

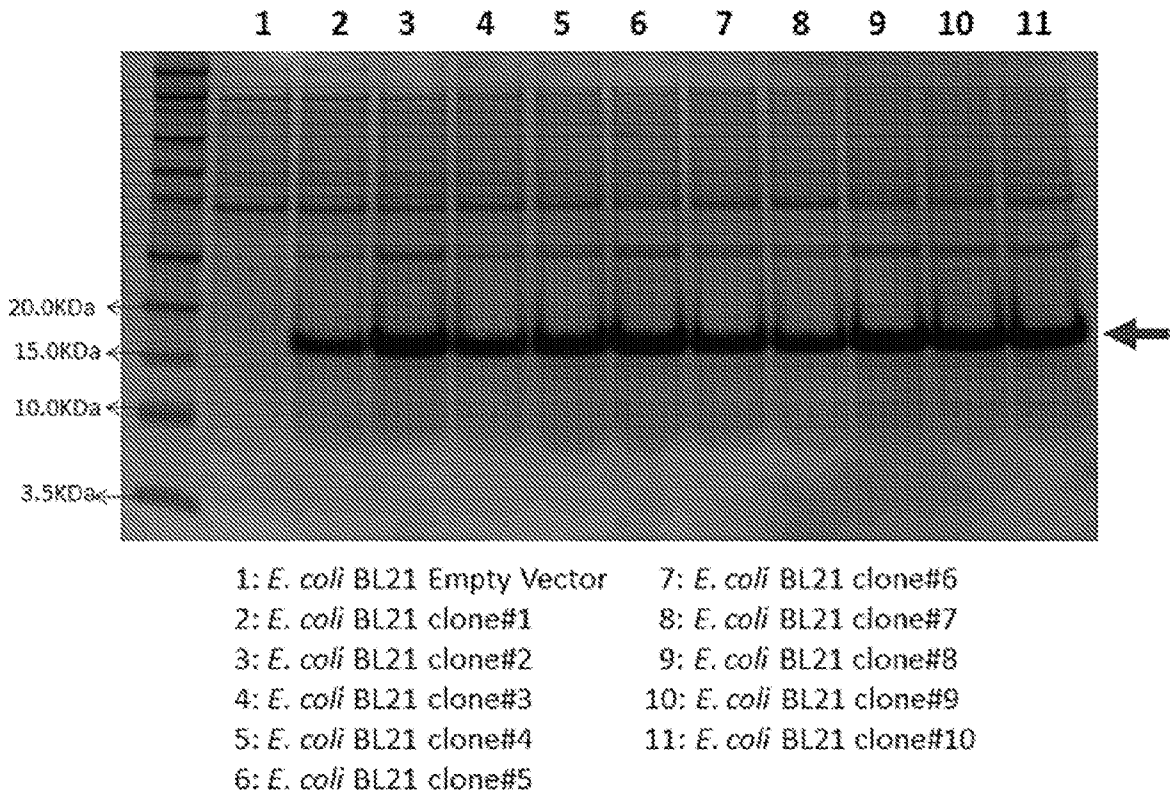


FIG. 8

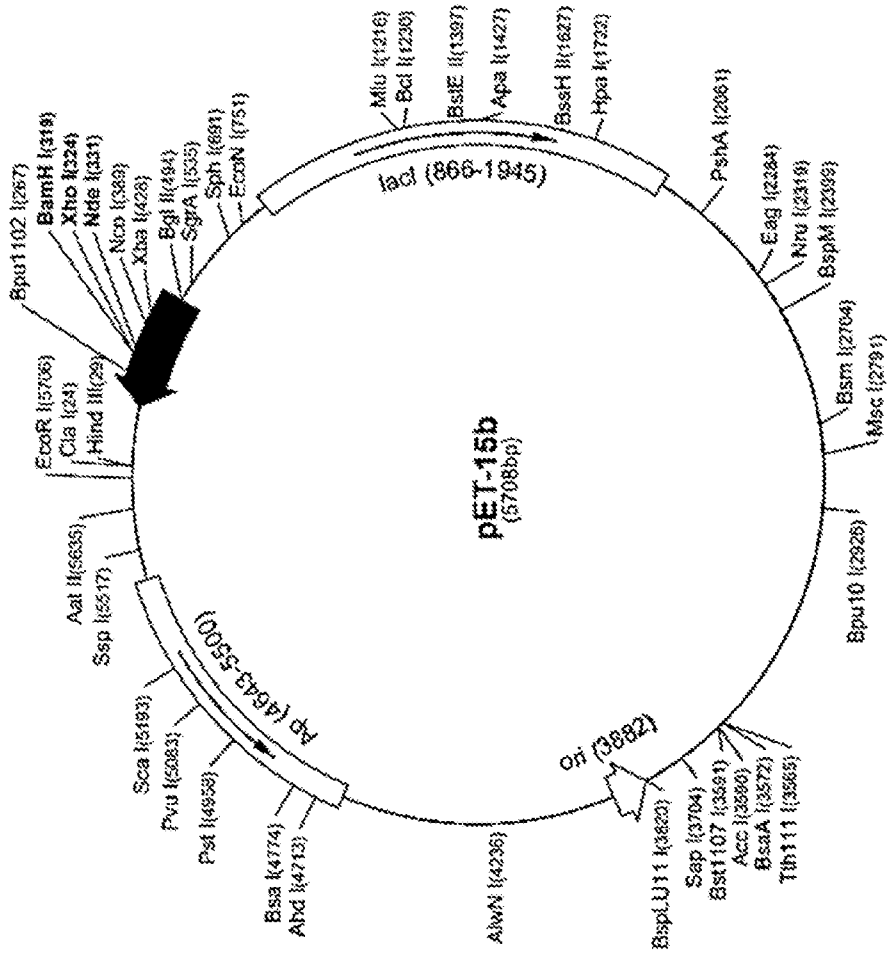


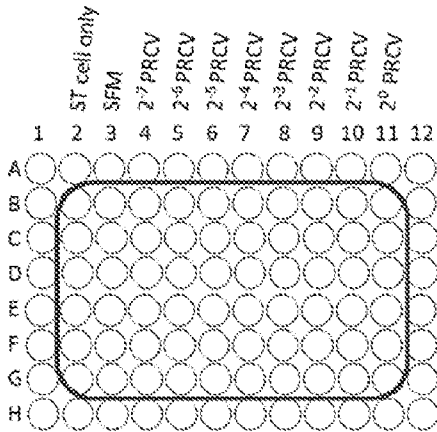
FIG. 9

| pET-15b sequence landmarks            |            |
|---------------------------------------|------------|
| T7 promoter                           | 453-463    |
| T7 transcription start                | 452        |
| His-Tag coding sequence               | 362-380    |
| Multiple cloning sites (Nde1 - BamH1) | 318-335    |
| T7 terminator                         | 213-239    |
| lacI coding sequence                  | (866-1945) |
| pBR322 origin                         | 3882       |
| msa coding sequence                   | 4843-5586  |



After 60 Hours Coincubation

Plate A

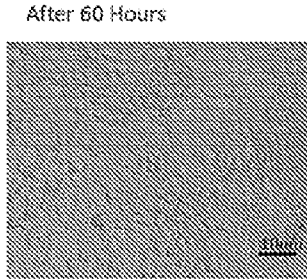


Only use center wells for the Test

Plate A: (No Protection Control)

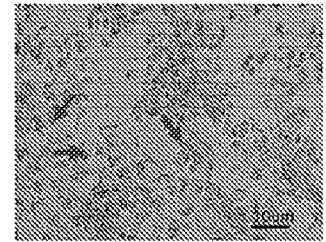
A. Virus particles only (Serial Dilution)

1. Seed Cell 80-90% confluent
2. Add Serum Free Medium (SFM) 100ul
3. Add PRCV 100uL (Serial Dilution)
4. Incubate 4 days
5. MTT Assay



ST cell (AB2)

Red Arrow: CPE (Cytopathic Effect)

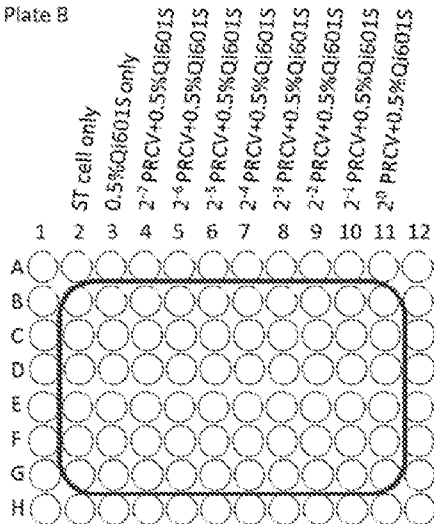


ST cell / PRCV (AB11)

FIG. 11

After 60 Hours Coincubation

Plate B

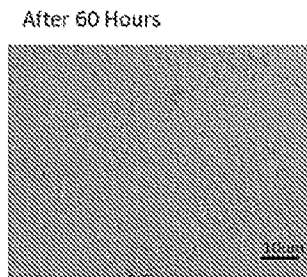


Only use center wells for the Test

Second Plate (0.5% Qj601S Protection)

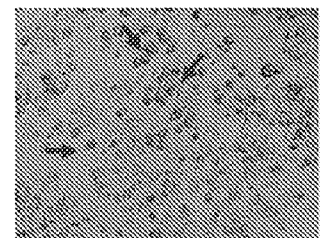
B. 0.5% Qj601S + Virus particles (Serial Dilution)

1. Seed Cell 80-90% confluent
2. Add 0.5% Qj601S 100ul
3. Add PRCV 100ul (Serial Dilution)
4. Incubate 4 Days
5. MTT Assay



ST cell (BB2)

Red Arrow: CPE (Cytopathic Effect)



ST/PRCV/0.5%Qj601S (BB11)

FIG. 12

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After 60 Hours Coincubation

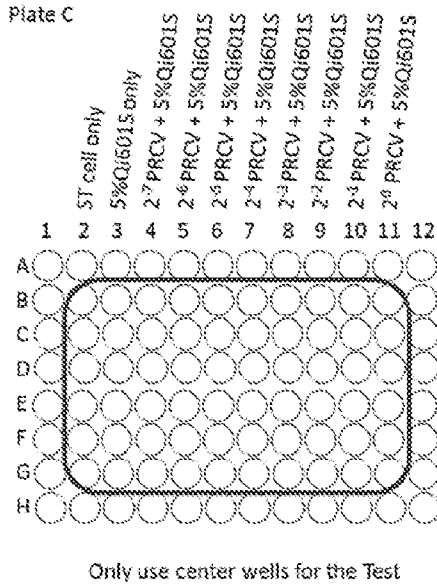
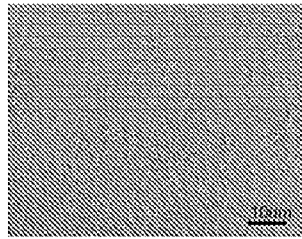


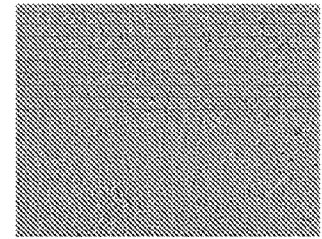
Plate C : (5% Qj601S Protection)  
 C. 5% Qj601S + Virus particles (Serial Dilution)

1. Seed Cell 80-90% confluent
2. Add 5% Qj601S 100ul.
3. Add PRCV 100ul (Serial Dilution)
4. Incubate 4 Days
5. MTT Assay

After 60 Hours



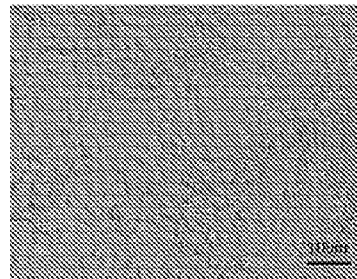
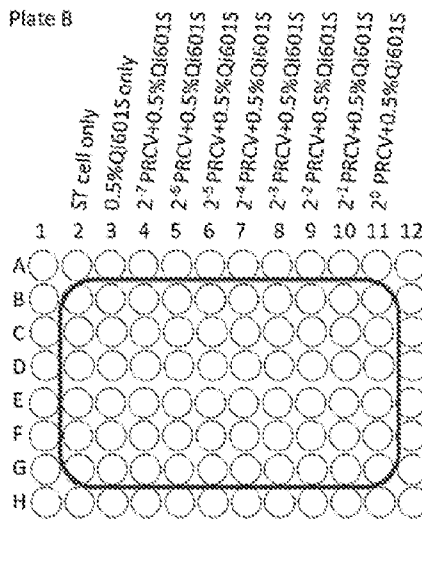
ST cell (CB2)



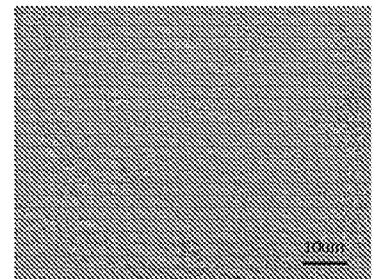
ST cell / PRCV / 5% Qj601S (CB11)

FIG. 13

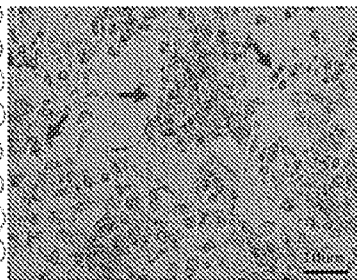
After 60 Hours Coincubation



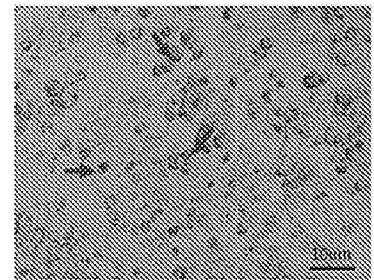
Normal ST cell (AB2)



ST cell / 0.5% Qj601S (BB3)



ST cell / PRCV (AB11)



ST/PRCV/0.5% Qj601S (BB11)

FIG. 14

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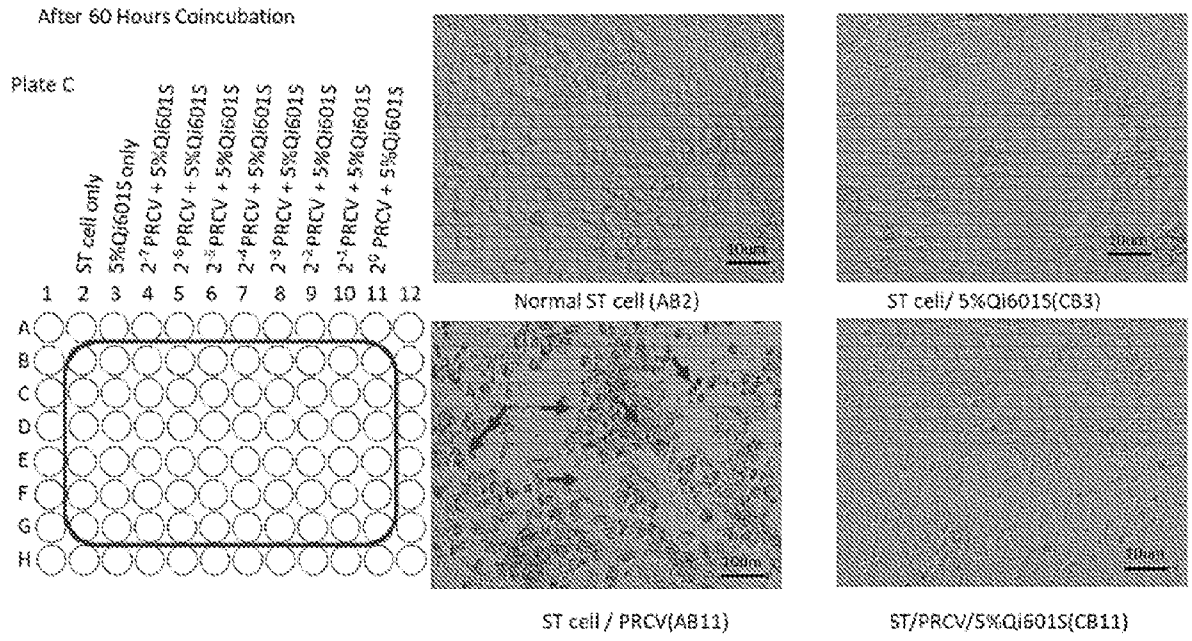


FIG. 15

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/036104

| <b>A. CLASSIFICATION OF SUBJECT MATTER</b><br>A61K 38/16(2006.01); A61K 35/747(2014.01); A61P 31/14(2006.01)<br><br>According to International Patent Classification (IPC) or to both national classification and IPC  |   |  |
|--|---|--|
| <b>B. FIELDS SEARCHED</b><br>Minimum documentation searched (classification system followed by classification symbols)<br>A61K 38/16(2006.01); A61K 35/74(2006.01); A61K 9/06(2006.01); A61K 9/107(2006.01); A61P 31/12(2006.01); C12N 1/20(2006.01)<br><br>Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched<br>Korean utility models and applications for utility models<br>Japanese utility models and applications for utility models<br><br>Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)<br>eKOMPASS(KIPO internal) & Keywords: Lactobacillus fermentum, Qi6, LfQi6, decontaminating, surface, antiviral, viral infection, inhibit, bacterial strain, biofilm   |   |  |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>  |   |  |
| Category*  | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No.  |
| X  | WO 2017-015275 A1 (QUORUM INNOVATIONS, LLC) 26 January 2017 (2017-01-26)<br>claims 1-2, 13, 31-32, pages 13-15  | 22   |
| A  | NCBI, Genbank accession no. WP_003682205.1 (2016.10.31)<br>the whole document   | 22   |
| A  | WO 2012-141540 A2 (CJ CHEILJEDANG CORPORATION) 18 October 2012 (2012-10-18)<br>the whole document   | 22   |
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| A  | WO 2007-040445 A1 (PROBI AB) 12 April 2007 (2007-04-12)<br>the whole document   | 22   |
| <input type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> See patent family annex.   |   |  |
| * Special categories of cited documents:<br>"A" document defining the general state of the art which is not considered to be of particular relevance<br>"D" document cited by the applicant in the international application<br>"E" earlier application or patent but published on or after the international filing date<br>"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)<br>"O" document referring to an oral disclosure, use, exhibition or other means<br>"P" document published prior to the international filing date but later than the priority date claimed<br>"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<br>"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<br>"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<br>"&" document member of the same patent family |   |  |
| Date of the actual completion of the international search<br><b>01 October 2021</b>  |   | Date of mailing of the international search report<br><b>01 October 2021</b> |
| Name and mailing address of the ISA/KR<br><b>Korean Intellectual Property Office<br/>189 Cheongsa-ro, Seo-gu, Daejeon<br/>35208, Republic of Korea</b><br>Facsimile No. +82-42-481-8578  |   | Authorized officer<br><b>Jung, Da Won</b><br>Telephone No. +82-42-481-5373   |

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
  - a.  forming part of the international application as filed:
    - in the form of an Annex C/ST.25 text file.
    - on paper or in the form of an image file.
  - b.  furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
  - c.  furnished subsequent to the international filing date for the purposes of international search only:
    - in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
    - on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2.  In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: **1-21**  
because they relate to subject matter not required to be searched by this Authority, namely:  
Claims 1-21 pertain to a method for treatment of the human body by surgery or therapy(PCT Article 17(2)(a)(i) and Rule 39.1(iv)).
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**INTERNATIONAL SEARCH REPORT**  
**Information on patent family members**

International application No.

**PCT/US2021/036104**

| Patent document cited in search report |                 |    | Publication date (day/month/year) | Patent family member(s) |                 |    | Publication date (day/month/year) |
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