Title: ADMINISTRATION OF FACTORS NORMALLY PRESENT IN A MICROBIAL NICHE TO IMPROVE HEALTH

Abstract: Methods to modulate the microbiota for the improvement of health using host and host-derived factors have been developed. Compositions for the modulation of the microbiota for the improvement of health comprising host and host-derived factors are also described. In particular, methods for identifying or compositions comprising host and host-derived compositions that are present in the healthy host environment and that normally select for or support the growth of beneficial commensal microbes are described.
ADMINISTRATION OF FACTORS NORMALLY PRESENT IN A
MICROBIAL NICHE TO IMPROVE HEALTH

FIELD OF THE INVENTION

This invention relates to substances and methods to identify substances that modulate populations of microbes that inhabit a host and uses of the substances to promote health.

BACKGROUND OF THE INVENTION

Animals, including humans, host multitudes of microbes (collectively referred to as the host's microbiota) in anatomical locations including the mouth, esophagus, stomach, small intestine, large intestine, caecum, colon, rectum, vagina, skin, nasal cavities, ear, and lungs. These locations offer environments with varying conditions of pH, redox potential, presence of host molecules and secretions, water content, and contact with the immune system, among other factors, where intense competition among bacteria leads to adaptation to the specific environment. Furthermore, the host exerts selective pressure for preferential survival of health-promoting microbes, especially during the healthy state of the host. As a result, pathogenic microbes are eliminated and groups of bacterial commensals that promote health and that are adapted to the host environment are established. These can be generally referred to as beneficial commensal microbes. Elucidation of the functional roles of such niches has been the focus of recent research, which has established that, collectively, the human microbiota is responsible for a multitude of critical processes, including metabolism of carbohydrates and proteins, maturation of the immune system, formation and regeneration of the epithelium, fat storage, production of hormones, metabolism of xenobiotics, production of vitamins, and protection from pathogen infections, among others (Hooper LV, Gordon JI. Science. 2001;292:1 115; Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Cell. 2004; 118:229; Backhed F, et al. Proc. Natl. Acad. Sci. U.S.A. 2004; 101 :1571 8; Stappenbeck TS, Hooper LV, Gordon JI. Proc. Natl. Acad. Sci. U.S.A. 2002;99:15451;l Sonnenburg JL, Angenent LT, Gordon JI. Nat. Immunol. 2004;5:569; Hooper LV, et al. Science. 2001;291 :881)

Despite the key importance of the microbiota, there is a lack of approaches to modulate it, and the few approaches available are inadequate.
Interventions known to modulate the microbiota are limited to antibiotics, prebiotics, probiotics, and synbiotics. Antibiotics generally eradicate the microbiota without selectivity as a byproduct of targeting an infectious pathogen. In contrast, nutritional approaches involving live organisms (probiotics), non-digestible food ingredients that stimulate the growth or activity of bacteria (prebiotics), or combinations of both (synbiotics), are more benign but exert a moderate beneficial effect on the host.

Antibiotics have been developed to inhibit or kill specific pathogens, and these antibiotics target the microbes and not the host. Prebiotics have been developed with the aim of promoting the growth of commensal organisms, but have relied on exogenous dietary fibers or dietary fiber derivatives to do so. Probiotics have been used with the aim of shifting the microbiota by supplying the microbes themselves, instead of by shifting the environment inhabited by the commensal organisms. Synbiotics have been developed that are mixtures of traditional prebiotics and probiotics. None of the microbiota modulators developed to date have contemplated the possibility of utilizing host factors normally present in the healthy host microbial niche, or derivatives of these factors, as microbiota modulators.

It is therefore an object of the present invention to provide formulations suitable for specific promotion of beneficial commensal organisms comprising host and host-derived factors, which enable modulation of commensal microbiota by shifting the environment towards a healthy host environment.

SUMMARY OF THE INVENTION

Methods to modulate the microbiota for the improvement of health using host and host-derived factors have been developed, as well as methods to identify such modulators. Compositions for the modulation of the microbiota for the improvement of health comprising host and host-derived factors are also described. In particular, compositions comprising host and host-derived factors that are present in the healthy host environment and that normally select for or support the growth of beneficial commensal microbes and methods for identifying the compositions are described. A general method for identifying modulators of the microbiota comprises (i) selecting a microbial niche (ii) identifying host compositions in that niche (iii)
purifying, synthesizing, or otherwise producing such compositions or derivatives of such compositions (iii) testing those compositions for microbiota modulating selectivity, and (iv) selecting a composition with beneficial microbiota modulating activity. A general method for modulating the microbiota may additionally comprise the steps of (v) administering such compositions with or without pharmaceutically acceptable carriers.

In one aspect, the improvement of health is selected from gut health, oral health, skin health, vaginal health, nasal health, pulmonary health, metabolic health, vascular health, endocrine health, immune health, or neurological health. In another aspect of the invention, the niche is in an animal. In another aspect the niche is an organ such as the gut, lungs, mouth, nose, esophagus, vagina, or skin. In another aspect, the niche is a non-gut niche.

In one aspect, the host compositions are present in the microbial niche in a healthy state. In yet another aspect, the host compositions are constitutively present or produced. In yet another aspect, the natural host compositions are abundant. In another aspect, the host compositions are a significant fraction of the host tissue in the niche, by mass. In another aspect, the host compositions are secreted. In another aspect, the host compositions are structural.

In one aspect, the compositions act on a microbial target. In yet another aspect, the natural host composition is able to be metabolized by the modulated members of the microbiota as an energy source. In yet another aspect, the natural host composition is toxic to members of the microbiota.

In yet another aspect, the natural host composition binds to the surface of members of the microbiota. In another aspect, the compositions act on a host target. In another aspect, the compositions are used to shift a chemical property of the host environment towards a normal healthy state (e.g., pH or salt concentration).

In another aspect, the compositions are selected from peptides, proteins, carbohydrates, lipids, nucleic acids, and small molecules. In yet another aspect the compositions are purified, recombinantly produced, or synthetically produced natural host compositions. In yet another aspect, the modulators are chemically modified natural host compositions. In yet
another aspect, the modulators are synthetic mimics of natural host compositions.

In another aspect, the compositions are tested for microbiota modulating activity using *in vitro* tests. In another aspect, these tests are conducted either with cultures of an individual organism or with co-cultured organisms. In another aspect, these tests are conducted in solid cultures or in solution cultures. In another aspect, the solution cultures are conducted in single compartment models or multi-compartment models. In another aspect, the tests are conducted in closed cultures or in actively maintained cultures. In another aspect the cultures are conducted using tissue explants. In another aspect, the tests measure preferential or increased growth of a certain microbe or group of microbes. In another aspect, the tests measure preferentially decreased growth of a microbe or a certain group of microbes. In another aspect, the test measures expression of at least one microbial gene. In another aspect, the tests measure expression of at least one microbial protein. In another aspect, the tests measure at least one microbial metabolite. In yet another aspect, the tests are conducted *in vivo*. In one aspect, the tests are conducted in an animal such as a mouse, rat, dog, cat, pig, chicken, or human. In one aspect, the test outcome is the improvement of a disease state or another physiological parameter. In another aspect, the test outcome is the change of a biochemical parameter.

In yet another aspect, the compositions are formulated for topical, subcutaneous, oral, intramuscular, intravenous, intranasal, inhaled, or rectal delivery. In another aspect, the compositions are formulated with pharmaceutically acceptable carriers.

In certain aspects, the composition is formulated in unit dosage form (e.g., a tablet, chewable tablet, caplet, hard capsule, soft capsule, or sachet). The unit dosage form can be a nutritional supplement. In particular aspects, the composition is a foodstuff (e.g., a baked good, beverage, beverage mix, food bar, biscuit, dairy product, candy, or other foodstuff.)
DETAILED DESCRIPTION

DEFINITIONS

The term "host factor" refers to a molecule present in a host tissue or biological fluid, such as a peptide, protein, carbohydrate, lipid, nucleic acid, or small molecule, which is part of the natural composition of the host tissue or biological fluid.

The term "host-derived factor" refers, collectively, to host factors and host factors modified by methods known in the art (e.g., medicinal chemistry, protein engineering, etc.) to be more efficacious in their intended use.

The term "microbiota" refers, collectively, to the entirety of microbes found in association with a higher organism, such as a human. Organisms belonging to a human's microbiota may generally be categorized as bacteria, archaea, yeasts, and single-celled eukaryotes, as well as various parasites such as Helminths.

The term "commensal" refers to organisms that are normally harmless to a host, and can also establish mutualistic relations with the host. The human body contains about 100 trillion commensal organisms, which have been suggested to outnumber human cells by a factor to 10.

The term "anatomical niche" describes a group of organisms, such as microbes, that populate a region of a host, such as the gut, the oral cavity, the vagina, the skin, the nasal cavities, or the lungs. The term may also refer to a structure or sub-region within any of these regions, such as a hair follicle or a sebaceous gland in the skin.

The term "functional niche" describes a group of organisms, such as microbes, that specialize in a certain function, such as carbohydrate metabolism or xenobiotic metabolism.

As used herein, the term "isolated" refers to a microbial modulator in a composition as described herein, wherein the microbial modulator is either (i) synthetically derived or (ii) isolated from a naturally occurring source such that the microbial modulator contains less than 15%, 10%, 5%, 4%, 3%, 2%, or 1% by mass other constituents present in the natural source and included in the isolated fraction or (iii) isolated from recombinant sources such as recombinant bacterial fermentation sources. The microbial
modulator can be prepared from naturally occurring sources using separation techniques known in the art, such as by precipitation, distillation, using chromatographic techniques, and combinations thereof.

The term "modulating" as used in the phrase "modulating a microbial niche" is to be construed in its broadest interpretation to mean a change in the representation of microbes or microbial activity in a bacterial niche of a subject. The change may be an increase or a decrease in the presence of a particular species, genus, family, order, class, or phylum. The change may also be an increase or a decrease in the activity of an organism or a component of an organism, such as a bacterial enzyme, a bacterial antigen, a bacterial signaling molecule, or a bacterial metabolite. The change may also be the change in the relative ratio of one bacterial species, genus, family, order, class, phylum, or component in relation to another.

The term "metagenomics" refers to genomic techniques for the study of communities of microbial organisms directly in their natural environments, without requiring isolation and lab cultivation of individual species.

The term "probiotic" refers to a dietary supplement of live microorganisms thought to be healthy for the host organism.

The term "prebiotic" refers to non-digestible dietary components that can help foster the growth of microorganisms, which may lead to better health.

The term "synbiotic" refers to a supplement that contains both a prebiotic and a probiotic.

The terms "targeting" or "targeted", as used in the phrase "targeted delivery", refers to methods of delivery of a cargo, such as a drug, to an individual in a manner that increases the concentration of the cargo in certain zones of the body relative to others.

**DISEASES AND CONDITIONS ASSOCIATED WITH THE MICROBIOTA**

Disease states may exhibit either the presence of a novel microbe(s), absence of a normal microbe(s), or an alteration in the proportion of microbes.
Recent research has established that disruption of the normal equilibrium between a host and its microbiota, generally manifested as a microbial imbalance, is associated with, and may lead to, a number of conditions and diseases. These include Crohn's disease, ulcerative colitis, obesity, asthma, allergies, metabolic syndrome, diabetes, psoriasis, eczema, rosacea, atopic dermatitis, gastrointestinal reflux disease, cancers of the gastrointestinal tract, bacterial vaginosis, neurodevelopmental conditions such as autism spectrum disorders, and numerous infections, among others. For example, in Crohn's disease, concentrations of Barteri hides, Eubacteria and Peptostreptococcus are increased whereas Bifidobacteria numbers are reduced (Linskens et al., Scand J Gastroenterol Suppl. 2001;(234):29-40); in ulcerative colitis, the number of facultative anaerobes is increased. In these inflammatory bowel diseases, such microbial imbalances cause increased immune stimulation, and enhanced mucosal permeability (Sartor, Proc Natl Acad Sci 71 S A. 2008 Oct 28; 105(43): 1641 3-4). In obese subjects, the relative proportion of Bacteroidetes has been shown to be decreased relative to lean people (Ley et al., Nature. 2006 Dec 21;444(7122):1022-3), and possible links of microbial imbalances with the development of diabetes have also been discussed (Cani et al., Pathol Biol (Paris). 2008 Jul;56(5):305-9). In the skin, a role for the indigenous microbiota in health and disease has been suggested in both infectious and noninfectious diseases and disorders, such as atopic dermatitis, eczema, rosacea, psoriasis, and acne (Holland et al. Br. J. Dermatol. 96:623-626; Thomsen et al. Arch. Dermatol. 116:1031-1034; Till et al. Br. J. Dermatol. 142:885-892; Paulino et al. J. Clin. Microbiol. 44:2933-2941). Furthermore, the resident microbiota may also become pathogenic in response to an impaired skin barrier (Roth and James Annu Rev Microbiol. 1988;42:441-64). Bacterial vaginosis is caused by an imbalance of the naturally occurring vaginal microbiota. While the normal vaginal microbiota is dominated by Lactobacillus, in grade 2 (intermediate) bacterial vaginosis, Gardnerella and Mobiluncus spp. are also present, in addition to Lactobacilli. In grade 3 (bacterial vaginosis), Gardnerella and Mobiluncus spp. predominate, and Lactobacilli are few or absent (Hay et al., Br. Med. J., 308, 295-298, 1994).
Other conditions where a microbial link is suspected based on preliminary evidence include rheumatoid arthritis, multiple sclerosis, Parkinson's disease, Alzheimer's disease, and cystic fibrosis.

**METHODS TO IDENTIFY HOST FACTORS IN A MICROBIAL NICHE**

In one embodiment, host factors for the modulation of the microbiota are identified by proteomic methods. In one embodiment, the factors are identified by multidimensional separation of polypeptides by two-dimensional gel electrophoresis (2-DE) or by multidimensional liquid chromatography (multi-LC) from a physiological complex mixture, and then identified by mass spectrometry, MALDI-TOF or ESI-MS/MS. In 2-DE, proteins are separated according to their isoelectric point in the first dimension and to their molecular weight in the second. In LC, proteins or peptides may be separated by any of a number of characteristics including hydrophobicity, degree of charge, or size. Typically, proteins in a complex mixture are separated by ionic and reverse phase column chromatography coupled to MS or MS-MS analysis. The complexity of the proteome and the separation limits of both 2D gel electrophoresis and liquid chromatography allow only a fraction of that proteome to be analysed. An alternative approach is to reduce the complexity of the protein sample prior to protein separation and characterization.

In one embodiment, host factors for the modulation of the microbiota are identified by genomic methods. Numerous methods exist in the art for sequencing DNA. For example, large-scale sequencing of very long DNA pieces, such as whole chromosomes may be performed by cutting with restriction enzymes or shearing with mechanical forces large DNA fragments into shorter DNA fragments. The fragmented DNA is cloned into a DNA vector, and amplified into a host such as *E. coli*. Short DNA fragments purified from individual bacterial colonies are individually sequenced and assembled electronically into one long, contiguous sequence.

In another embodiment, "gene chips" containing an array of genes that respond to extracted mRNAs produced by cells (Klenk et al., Nature. 1997 Aug 7;388(6642):539-47) can be used. Several genes can be placed on a chip array and patterns of gene expression, or changes therein, can be
monitored. In another embodiment, Reverse transcription polymerase chain reaction (RTPCR) can be used to monitor specific RNA expression.

In one embodiment, host factors for the modulation of the microbiota arc idenfied by metabolomic or metabonomie approaches. These methods have been developed to complement the information provided by genomics and proteomics by analyzing metabolite patterns (See, for example, Nicholson et al., 1999). Metabonomics is based on the application of IH NMR spectroscopy and mass spectrometry to study the metabolic composition of biofluids, cells, and tissues, in combination with use of pattern recognition systems and other chemoinformatic tools to interpret and classify complex NMR-generated metabolic data sets.

In another embodiment, host factors for the modulation of the microbiota are idenfied by "Glycomic" methods. These methods can be used to comprehensively study glycomes (the entire complement of sugars, whether free or present in more complex molecules, of an organism). The tool used most often in glycomic analysis is high resolution mass spectrometry. In this technique, the glycan part of a glycoprotein is separated from the protein and subjected to analysis by multiple rounds of mass spectrometry. Mass spectrometry can be used in conjunction with HPLC. Other techniques include lectin and antibody arrays, as well as metabolic and covalent labeling of glycans.

In another embodiment, host factors for the modulation of the microbiota are identified by "lipidomic" approaches. Lipid profiles pertaining to biological networks of the invention can be studied with a number of techniques that rely on mass spectrometry, nuclear magnetic resonance, fluorescence spectroscopy and computational methods. The techniques involve steps of lipid extraction (using solvents well known in the art), lipid separation (typically using Solid-phase extraction (SPE) chromatography, and lipid detection (typically using soft ionization techniques for mass spectrometry such as electrospray ionization (ESI) and matrix-assisted laser desorption/ ionization (MALDI)
HOST COMPOSITIONS IN MICROBIAL NICHES THAT MAY BE USED TO MODULATE MICROBIOTA

Host compositions derived from the skin

The skin harbors commensal microbiota which differ depending on the anatomical location (for example the arm, or the scalp). The environmental conditions of the anatomical locations may vary widely in their range of pH, temperature, moisture, and sebum content. Furthermore, within a certain anatomical location dissimilar local microbiota compositions may exist in association with subhabitats of the anatomical location (such as the hair follicle, the sweat glands, and the sebaceous glands in the case of skin).

In one embodiment, the composition is derived from the stratum corneum. In another embodiment, the composition is derived from the epidermis. In another embodiment, the composition is derived from the dermis. In another embodiment, the composition is derived from sweat gland secretions. In another embodiment, the composition is derived from sebaceous gland secretions. In another embodiment, the composition is derived from melanocytes. In another embodiment, the composition is derived from fibroblasts. In another embodiment, the composition is derived from immune cells.

In yet another embodiment, the composition is comprised of compositions derived from keratin, collagen, other skin proteins, sebum, lipids, triglycerides, diglycerides, free fatty acids, wax esters, squalene, cholesterol, cholesterol esters, DNA, carbohydrates, hyaluronic acid, heparin or heparan sulfate, chondroitin sulfate, or dermatan sulfate. In yet another embodiment, the composition further comprises pharmaceutically acceptable delivery vehicles, diluents, buffers, or excipients. In yet another embodiment, the compositions contain alcohol.

In yet another embodiment, the compositions modulate the microbiota by decreasing harmful microbial species. In yet another embodiment, the compositions modulate the microbiota by decreasing levels of certain microbes, including fungi, Candida species, Escherichia species, Pseudomonas species, and Staphylococcus species. In yet another embodiment, the compositions modulate the microbiota by increasing the
prevalence of certain microbes, including Staphylococcus, Micrococcus, Corynebacterium, Brevibacterium, Propionibacteria, and Acinetobacter spp, or a specific species, such as Propionibacterium acnes.

In another embodiment, the compositions promote a function of the skin microbiota selected from inhibition of pathogenic species, processing of skin proteins, processing of skin free fatty acids, and processing of sebum.

In another embodiment, the compositions further comprise molecules derived from the skin microbiota. Such molecules may comprise bacteriocins (such as hemolysins and lantibiotics), and quorum-sensing molecules (such as phenol soluble modulins).

In one embodiment, the compositions are topically administered. The topical formulations used to apply the compositions may comprise a cream, a lotion, a gel, a patch, an ointment, a salve. In another embodiment, the formulations are administered by iontophoresis. The formulations may also be delivered by skin microneedles and fluid jets or other known mechanisms for penetrating the skin, all of which have been extensively described in the art.

In yet another embodiment, the improvement of skin health is the treatment of a disorder comprising psoriasis, eczema, atopic dermatitits, keratosis, pemphigus, rosacea, pigment disorders, dry skin, xerosis, vitiligo, wrinkles, scars, burns, blisters, acne, hirsutism, and alopecia.

**HOST COMPOSITIONS DERIVED FROM THE MOUTH**

In one embodiment, the compositions are modulators for oral health. The mouth harbors commensal microbiota which differ depending on the anatomical location. The supragingival plaque (dental plaque) is covered by multiple types of microbial biofilms, such as biofilms on the surface of teeth above the gingival crevice. The subgingival plaque, the tongue, mucosal surface of buccal cells and the floor of the mouth, and dental prosthetic and fillings can also host microbial biofilms, as has been described in the art (Jenkinson and Lamont, Trends Microbiol. 2005 Dec; 13(12):589-95; Marsh, Int Dent J. 2006 Aug;56(4 Suppl 1):233-9).

In one embodiment, modulators are derived from host factors in the saliva, the components of which have been extensively reviewed (de Almeida et al., J of Contemp Dent Pract. 2008, 9(3): 72-80). In one
In one embodiment, the modulator contains salivary electrolytes (sodium, potassium, calcium, chloride, magnesium, bicarbonate, phosphate, fluoride). In one embodiment, the modulator contains salivary proteins selected from enzymes, immunoglobulins, antimicrobial factors, mucosal glycoproteins, albumin, and polypeptides and oligopeptides. In one embodiment, the modulators contain salivary small molecules such as glucose and nitrogenous products, such as urea and ammonia.

In one embodiment, the host components are derived from the salivary glands, the gingival fold, oral mucosa transudate, the mucous of the nasal cavity and pharynx, food remainders, or desquamated epithelial and blood cells. In another embodiment, the modulators are derived from salivary mucins, which can modulate the adhesion of microorganisms and protect tissues against proteolytic attack.

In one embodiment, modulators consist of the buffering components of saliva which neutralize acids produced by acidogenic microorganisms. These buffering components include but are not limited to phosphate, carbonic acid-bicarbonate, negatively charged amino acid residues, salivin, and urea. Urea causes a rapid increase in biofilm pH when hydrolyzed by bacterial ureases into ammonia and carbon dioxide. In yet another embodiment, the modulator consists of ammonia.

In another embodiment, the modulator consists of salivary molecules which affect or are affected by calcium such as fluoride, citrate, statherin, histidine-rich peptides, proline rich peptides, and alpha-amilase.

In yet another embodiment, the modulator consists of digestive enzymes such as alpha-amylase (ptyalin).

In yet another embodiment, the modulator consists of salivary digestive products such as maltose, maltotriose, and dextrins. In yet another embodiment, the modulator consists of immunologic or non-immunologic proteins with anti-bacterial properties. These molecules may comprise secretory immunoglobulin A (IgA), Immunoglobulin M, Immunoglobulin G, enzymes (lysozyme, lactoferrin, and peroxidase or sialoperoxidase), mucin glycoproteins, agglutinins, histatins, proline-rich proteins, statherins, and cystatins. The molecules perform a host of beneficial functions: lactoferrin causes bactericidal or bacteriostatic effects on various microorganisms such
as the \textit{Streptococcus mutans} group, and it also provides fungicidal, antiviral, anti-inflammatory, and immunomodulatory functions. Peroxidase or sialoperoxidase has antimicrobial activity. The proline-rich proteins and statherins selectively mediate bacterial adhesion to tooth surfaces. The cystatins play functions in film formation and in controlling proteolytic activity. The histatins have antimicrobial activity against some strains of \textit{Streptococcus mutans}, inhibit hemoagglutination of the periopathogen \textit{Porphyromonas gingivallis}, and are potent inhibitors of \textit{Candida albicans} growth and development. Salivary agglutinin is one of the main salivary components responsible for bacteria agglutination (See de Almeida \textit{et al}, \textit{J Contemp Dent Pract}, 2008 Mar 1; 9 (3): 72-80).

In another embodiment, the modulator is a salivary gel-forming mucin, preferably MUCB5. MUC5B is a nutrient for dental plaque bacteria, and can constitute a major source of nutrients for the oral supragingival microbiota during long periods of starvation between meals (Wickstrom \textit{et al}, \textit{Oral Microbiology and Immunology}, 23 (3), 177-182, 2008). The modulator may act on selected members of the resident oral microbiota that are able to degrade it and use it as a nutrient source, including \textit{Streptococcus oralis}, \textit{Streptococcus intermedins}, \textit{Streptococcus mitis}, and \textit{Actinomyces naeslundii}. In another embodiment, the modulator is MUC7.

In one embodiment, the modulators are derived from or are more similar to parotid gland saliva. In one embodiment, the modulators are derived from or are more similar to submandibular gland saliva. In one embodiment, the modulators are derived from or are more similar to sublingual gland saliva. In one embodiment, the modulators are derived from serous, mucous, or mixed secretions.

In another embodiment, the improvement of oral health is the treatment of a condition comprising chronic gingivitis, periodontitis, caries, and endodontic and restoration failures.

In another embodiment, the compositions modulate the microbiota by increasing the prevalence of at least one of \textit{Streptococcus oralis}, \textit{S. cristatus}, \textit{s. gordonii}, \textit{Veillonella spp.}, \textit{Actinomyces naeslundii}, \textit{Capnocytophaga} spp. and \textit{Leptotrichia} spp.
The compositions may be administered topically directly to the buccal epithelium. The compositions may also be administered to the mouth of an animal by incorporating them into an adhesive patch that is applied on the mucosal surface of buccal cells, on the floor of the mouth, on the tongue, or on the surface of the teeth. Alternatively, the compositions may be administered via a dental prosthetic, for example by coating the surface of a dental prosthetic with a carbohydrate modulator of the oral microbiota that dissolves under the action of carbohydrases in the saliva. The compositions may also be administered via a floss, for example by coating the surface of the floss with a modulator that can be detached from the floss under shear or under the action of salivary enzymes. The compositions may also be administered in a food, gum, candy, beverage, or wash.

**HOST COMPOSITIONS DERIVED FROM THE AIRWAYS**

In one embodiment, the compositions are modulators for pulmonary, hmg, or airway health. Some areas of the upper airway have an environment that sustains commensal microbes. For example, the oropharynx is colonized by a microbiota that can be altered by infections and by alcohol intake (Golin et al, Sao Paulo Med J, 116 (3), 1998).

In one embodiment, the modulators are administered by dry powder inhalation or vapor inhalation. In one embodiment, the modulators are administered by gavage. In a preferred embodiment, the modulators are formulated into aerosols. Aerosols for the delivery of therapeutic agents to the respiratory tract have been described, for example, Adjei, A. and Garren, J. Pharm. Res., 7: 565-569 (1990); and Zanen, P. and Lamm, J.-WJ. Int. J. Pharm., 114: 111-1 15 (1995). Dry powder formulations with large particle size and improved flowability characteristics, such as less aggregation have been described (Visser, J., Powder Technology 58: 1-10 (1989)). The modulators may be contained in a porous particle suitable for aerosolization in a dry powder inhaler; ideally, the particle may have a density less than about 0.4g/cm³ and an aerodynamic diameter of less than 4 microns.

Analysis of epithelial lining fluid (ELF) in the airways has been conducted by the study of samples, such as cell cultures, biopsies and physiological fluids like serum and, especially, induction of sputum with hypertonic saline, nasal lavage, condensation of exhaled breath, and
bronchoalveolar lavage fluid (BALF). The airways and, particularly, the alveoli are covered with a thin layer of ELF, which functions by protecting the lung and preserving its gas-exchange capacity. In one embodiment, the modulators are derived from ELF. In one embodiment, the modulators are derived from proteins found in BALF comprising alpha-2-macroglobulin; ceruloplasmin; immunoglobulin A-S chain; prothrombin; 1-B-glycoprotein; hemopexin; complement factor B; plasminogen; transferring; complement c3; albumin; antiplasmin; immunoglobulin heavy chain; antithrombin III; Vitamin D-binding protein; alpha 1-antitrypsin; alpha 1-antichymotrypin; HSGlycoprotein; leucine-rich 2-glycoprotein; fibrinogen; orosomucoid 1; haptoglobin; Zn-2-glycoprotein; clusterin; 1-microglobulin; complement C4; pulmonary surfactant-associated protein A; apolipoprotein A-I; cathepsin D heavy chain; proapolipoprotein Al; serum amyloid P-component; serum retinol binding protein; superoxide dismutase (Cu-Zn); transthyretin; immunoglobulin binding factor; thioredoxin; 2-microglobulin; phosphatidylethanolamine binding protein; peroxisomal antioxidant enzyme; peptidyl-prolyl cis-trans isomerase A; calgranulin A; GTP cyclohydrolase I feedback regulatory protein; ubiquitin; haemoglobin.

In yet another embodiment, the proteins are common between ELF and plasma including alpha 1-antitrypsin, macroglobin, apolipoprotein Al, 2-microglobulin, ceruloplasmin, complement factors 3 and 4, and immunoglobulins. In yet another embodiment, the proteins are proteins that are lung specific, such as surfactant protein A (SP-A) and clara cell protein 16 (CC 16). In another embodiment, the protein is a heavily glycosylated protein such as a mucin.

In one embodiment, the modulators are derived from airway secretions such as bronchial and pharyngeal secretions, and may comprise IgA, bronchial lactoferrin, bronchial lysozyme, serum proteins, and mucin.

In one embodiment, the modulators are derived from carbohydrate constituents of airway secretions. The carbohydrates may be common constituents of mucin. They may comprise galactose, fucose, mannose, galactose, hexosamines, sialic acids, uronic acids chondrosamine, and glucosamine, among others.
In another embodiment, the improvement of pulmonary, lung, or airway health is the treatment of a condition selected from asthma, cystic fibrosis, an airway infection, or gastro-esophageal reflux disease. In another embodiment, the modulators are applied prophylactically to a subject to prevent an infection. Preferably, the patient is a heavy alcohol drinker (the airways microbiota has been shown to be altered by alcohol intake (Golin et al, Sao Paulo Med J, 116 (3), 1998). The compositions may modulate the microbiota of the airways by decreasing anaerobic bacteria (such as *Bacteroides* sp, *Prevotella melanin*, *Fusobacteriiim* sp., *Vellionella* sp., *Peptostreptococcus* sp., *Propionibacterium* sp., or *Bifidobacterium* sp., or *Clostridium* sp.), and fungi (such as *Candida* sp), or by decreasing *K. pneumoniae* or *Enterobacter* sp.) The compositions may also modulate the microbiota by increasing the prevalence of certain microbes, including *Staphylococcus* (for example, *S. aureus* and *S. epidermis*), *Streptococcus* (for example *S. viridians* and *S. pyogenes*), *Enterococcus* sp., *Neisseria* sp., and *Entereobacteria* (for example *Kleibsella* sp. And *E. coli*). In a preferred embodiment, the modulators decrease concentrations of *Candida* sp.
HOST COMPOSITIONS DERIVED FROM THE VAGINA

In one embodiment, the compositions are modulators for vaginal, cervical, gynecological, or reproductive health. The female reproductive tract supports commensal microbes. Dysbiosis in this niche can cause infection and yeast or fungal overgrowth, in some cases by invasion via the mucosa or the epithelial layer. Health repercussions of dysbiosis and infection can include preterm birth, infertility, increased susceptibility to sexually transmitted diseases and cancer, or more minor repercussions such as discomfort, odor, and discharge. Adherent bacteria are constantly removed by shedding epithelial cells, excretions from the cervical and vaginal glands, and by plasma transudate. Cervical-vaginal fluid (CVF) plays an important role in preventing infection and maintaining the homeostatic environment. CVF can be affected by menstruation, age, infection, sexual intercourse, usage of contraceptives, pregnancy, etc.

Commensal bacteria in the vaginal niche such as Lactobacillus spp. produce organic acids and compete with detrimental bacteria.

Samples of CVF may be obtained by a number of methods, including swabbing, biopsy, lavage, and colposcopy (which may require washing the vagina with approximately 5% acetic acid. The cervicovagina may be washed with 50 ml of 5% acetic acid for 2 minutes, the lavage fluid containing the CVF collected (15-30 ml), and immediately transported to the laboratory and stored at -80°C). Samples may be prepared for downstream analysis by methods known in the art.

Modulators may be derived from the epithelial cell membrane, the cornified envelope (a structure located below the epithelial cell membrane), involucrin, small proline rich proteins, cystatin A, periplakin, enveloplakin, annexin I, and desmosomal proteins. Modulators may also be derived from endometrial materials. These may comprise endometrial proteins such as glycodelin and ribonucleoprotein A. These may also comprise components from blood or blood cells. Modulators may also be derived from mucous and secretion-forming material, such as mucins, and carbonic anhydrase 1/2. Modulators may also be derived from plasma transudate or from CVF. The modulators may comprise immunological molecules present in the vaginal niche such as secretory IgA and IgG, neutrophil and eosinophil granule
secretion fluids, antimicrobial proteins/peptides (e.g., defensins, lactoferrin, cathelicidin, lysozyme, SLPI, beta-defensin-2, azurocidin, myeloperoxidase, TLR-7, IL-17, etc.). These molecules may sequester microbial nutrients, disrupt microbial proteins and membranes, and prevent microbial adhesion, as well as affect host immunity. Modulators may also be derived from proteins involved in protein metabolism and modification, in developmental processes, and signal transduction. Modulators may also be derived from proteins selected from cytoplasmic proteins, extracellular proteins, membrane proteins, or cytoskeletal proteins. The proteins may have serine protease activity. Modulators may be derived from proteases including kallikrein-6/10/1 1/13/14, transmembrane serine proteases 1/h/1 IE, leukocyte elastase and myeloblastin, and other proteases (e.g. cathepsin G). Modulators may also be derived from protease inhibitors, such as inhibitors of serine proteases including serpin B3/B4/B12/B1 3, calpastatin, SLPI, alpha-1 antitrypsin, serine protease inhibitor Kazal-type 7/5, and plasma serine protease inhibitor, or inhibitors of cysteine proteases including calpastatin and cystatin A/B, or inhibitors of other proteases such as SLPI, and WAP four-disulfide core domain protein. Modulators may also be derived from high-abundance proteins such as high-abundance immunological proteins S100A9 and S100A8, cystatin A/B, antileukoproteinase, immunoglobins and elafin. Modulators may also be derived from high-abundance plasma proteins such as serum albumin and hemoglobin alpha/beta, or from any proteins known in the art to be found in multiple proteomic studies of CVF, such as those referenced in Cole AM, Innate host defense of human vaginal and cervical mucosae. *Curr Top Microbiol Immunol* 2006, 306:199-230

Modulators may also be derived from vaginal lubrication characteristic of healthy vaginal activity, including pyridine, urea, squalene, and lactic acid.

In one embodiment, the products modulate the microbiota by decreasing harmful species. In another embodiment, the products modulate the microbiota by decreasing levels of certain microbes, including fungi, *Candida* species, *Escherichia* species, *Pseudomonas* species, and *Staphylococcus* species. In yet another embodiment, the products modulate...
the microbiota by increasing the prevalence of certain microbes, including *Lactobacillus acidophilus*, *Lactobacillus fermentum* *Lactobacillus brevis*, *Lactobacillus jensenii*, *Lactobacillus casei*, *Lactobacillus crispatus*, or *Lactobacillus jenseni* (See Appl Environ Microbiol. 2003 January; 69(1): 97-101 for a characterization of species that colonize the vagina)

**HOST COMPOSITIONS DERIVED FROM THE GUT**

In one embodiment, the compositions are modulators for gastrointestinal health. The human gut supports a great variety of commensal microbes (See for example Turnbaugh, P.J. et al., Nature 444:1027-1031 (2006)), and dysbiosis in this niche can have numerous health repercussions.

In one embodiment, the modulators are derived from sources of digestive secretions in the intestine, the major sources being the pancreas (which empties its secretions to the gut via the pancreatic duct) and the liver (which empties its secretions via the gallbladder. The modulators may comprise mucus, hormones, water, inorganic salts (alkaline salts such as sodium bicarbonate), bile acids (such as glycocholate, taurocholate, cholesterin, and lecithin), and organic material (containing cellular debris and enzymes, including pepsin-like proteases, amylases, lipases, peptidases, sucrases, maltases, enterokinases, alkaline phosphatases, nucelophasphatases, and nucleocytases).

In another embodiment, the improvement of gut health is the treatment of a disorder comprising Crohn's disease, ulcerative colitis, obesity, asthma, allergies, metabolic syndrome, diabetes, gastrointestinal reflux disease, cancers of the gastrointestinal tract, neurodevelopmental conditions such as autism spectrum disorders, and gastrointestinal infections.

In another embodiment, the compositions modulate the microbiota of the gut by decreasing concentrations of *Bacterioides*, *Eubacteria* and *Peptostreptococcus* in a Crohn's disease patient, or increasing concentration of *Bifidobacteria* in a Crohn's patient (dysbiosis of the bacterial populations in Crohn's disease has been established by Linskens et al., *Scand J Gastroenterol Suppl* 2001;234:29-40, 2001); In another embodiment the compositions increase the amount of *Faecalibacterium Prausnitzii* in Crohn's patients. In another embodiment, the compositions decrease the
number of facultative anaerobes in an ulcerative colitis patient. In another embodiment, the compositions increase the relative proportion of *Bacteroidetes* vs *Firmicutes* in an obese patient (dysbiosis of the bacterial populations in obese subjects has been established by Ley et al., *Nature* 444, 1022-1023, 2006).

**METHODS TO TEST COMPOSITIONS FOR MODULATING ACTIVITY TOWARDS MICROBIOTA**

Methods for evaluating the capability of fiber-based substances to be fermented by beneficial bacteria, to alter the metabolism of beneficial bacteria, or to be able to alter bacterial population towards a healthier composition (either by stimulating the growth of beneficial bacteria or by inhibiting the growth detrimental bacteria) have been described in the art (See for example US20070196890A1). Variations of the methods can be used to evaluate the capability of non-fiber-based substances (for example proteins, lipids, digestible carbohydrates, etc) to be metabolized by bacteria or to otherwise affect their viability or growth.

The methods may seek to determine a parameter or quantify a process undertaken by bacteria when exposed to a modulator, such as the growth rate, maximum growth rate, or number of bacteria, or the rate of assimilation of the modulator molecule by a certain bacteria, or the rate of production of a fermentation product of the modulator molecule by the bacteria.

In one embodiment, modulators of a bacterial niche are identified by (1) incubating a bacterial culture (which may be extracted from a human donor by any of the methods previously discussed, such as sampling of biofluids, swabs, biopsies, etc) in the presence and absence of a potential modulator; (2) measuring the amount and composition of bacteria in both cultures, and (3) comparing the measurements of amount and composition of bacteria with and without modulator. In another embodiment, methods to identify modulators can also comprise the step of measuring the relative ratios of certain bacteria in one or more cultures.

Methods to measure the amount, composition, growth rate, maximum growth rate of bacteria, as well as substrate consumption by bacteria may comprise anaerobic cultures containing the tested modulator or mixture of modulators, as well as an inoculum of a bacterial population of interest. The
cultures may be batch cultures, chemostat-type simulators, and non-chemostat-type simulators, all of which have been reviewed in the art (See *In vitro Methods to Model the Gastrointestinal Tract*, p 237-252, Chapter 12, in Gastrointestinal Microbiology, edited by A Ouwehand and E Vaughan)

**METHODS TO SELECT HOST MODULATORS BASED ON THE HOST MICROBIAL GENOMIC POTENTIAL**

The selection of host modulators can be guided by mining the host microbial metagenome for sequences coding proteins or peptides predicted to interact, directly or indirectly, with the candidate host modulators. The candidate host modulators can be selected to expand a beneficial microbial taxa or reduce a problematic taxa, based on the taxa genomic potential, rather than the specific identity of taxa itself.

In one embodiment the host microbial sequences mined for interacting with candidate modulators are chosen among genomic sequences predicted to facilitate nutrient acquisition and therefore cellular growth. Examples of genomic sequences involved in nutrient acquisition are those predicted to code for enzymes catalyzing the hydrolysis of O- or S-glycosides such as glycosidases, glycosyltransferases. The target sequences and predicted functions of these enzymes can be obtained by those skilled in the art on CAZy database (Carbohydrate-Active EnZymes). Common target nutrient acquisition enzymes useful as described herein are glycosidases and glycosyltransferases. In one embodiment the target sequences in the host microbiome are predicted to code for one or more of the following enzymes: alpha-L-Fucosidase, Amylase, beta-Fructofuranosidase, Chitinase, Dextranase, Disaccharidase, Galactosidase, Glucosidase, Glucoronidase, N-Glycosyl Hydrolase, Hyaluronoglucosaminidase, Isoamylase, Mannosidase, Muramidase, Neuraminidase, Polygalacturonase, Xylosidases.

The targets coding for nutrient acquisition enzymes can be mined in the host microbiome and based on their predicted substrate specificity a preferred cognate host modulator can be selected. A preferred cognate modulator is a nutrient, such as an oligosaccharide capable of being hydrolyzed into smaller units or individual monosaccharide moieties by nutrient acquisition enzymes. In some embodiments, the candidate host modulators are selected for their specificity towards nutrient acquisition
genes modulating cellular growth across evolutionarily-related groups such as a taxon, one or multiple species in a genus, one or multiple subspecies in a species. When the target nutrient acquisition genes are shared across functional groups of microorganism, such as in members exchanging genetic material by horizontal gene transfer, the cognate host modulators can be selected to modulate the functional attributes of the host microbiome.

In some methods, the host modulator is selected by comparing the metagenome of a test and a control host microbiome, identifying shared and divergent genetic loci, and selecting one or more desired loci based on their functionality or taxonomic placement. Examples of useful genetic loci are the polysaccharide utilization loci. In some methods, the desired loci, and therefore their corresponding taxonomic or functional units, can be enriched by administering the host microbiome with a cognate modulator, such an oligosaccharide, catalyzed by enzymes produced in the selected loci.

**METHODS TO ISOLATE OR PURIFY MODULATORS FROM TISSUE OR BIOFLUID SAMPLES**

Several techniques exist for the extraction and purification of biological samples, including solid-phase extraction (SPE), high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), size exclusion chromatography (SEC), immunoaffinity purification, microfluidic tools, selective precipitations, and combinations of the above.

Extraction of potential modulators from biological samples may be attained by solvent extraction methods, such as SPE, which may be particularly helpful for the extraction of small molecule metabolites. In general, small molecule metabolites can be extracted with reverse-phase cartridges specific to the molecules (e.g. See Lawson et al, Anal. Biochem, 150, 463-470. 1985), for cartridges to extract eicosanoids). Purification by SPE only is generally insufficient, and further chromatographic purification methods may be required to ensure adequate purity of the sample. Both reverse and normal-phase HPLC and TLC have been used in the art.

Modulators contained into tissues may be brought into solution by breaking the tissue or cells containing it, using methods such as repeated freezing and thawing, sonication, high pressure homogenization, clarification via cellulose-based depth filters, or permeabilization by organic solvents. Any...
of these techniques may be followed by centrifugation. Immunoaffinity methods may also be used to purify a potential modulator, wherein the modulator is extracted by an immobilized specific antibody.

Protein modulators may be separated from complex samples by exploiting differences in protein size, physico-chemical properties and binding affinity. Proteins may be purified according to isoelectric points by ion exchange chromatography or by running the proteins through a pH-graded gel. Proteins may also be further purified according to molecular weight, by using size exclusion chromatography, SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), and 2D-PAGE. Proteins may also be further separated by their hydrophobicity via HPLC or reversed-phase chromatography, all of which are well-known methods in the art. If the potential protein modulator is present in low abundance, recombinant expression systems coupled to a His-tag to facilitate production and purification may be used.

Carbohydrate modulators may be separated from complex biological samples using a range of techniques known in the art, such as solubilization by treatment with enzymes followed by precipitation by cationic surfactants such as cetyltrimethylammonium bromide, or followed by fractionation on weak anion exchangers (if the carbohydrates are negatively charged). If the carbohydrates have neutral charge, more elaborate precipitation protocols involving initial removal of proteins and nucleic acids may be used (See "Carbohydrate chemistry and biochemistry, Michael Sinnott, RSC Publishing, 2007"). Additionally, chromatographic methods, such as gas-liquid chromatography combined with mass spectrometry (GLC-MS), may be used. Anion-exchange chromatography coupled to pulsed amperometric detection (HPAEC-PAD) may also be used for sensitive detection of a carbohydrate, although the method destroys the carbohydrate molecule.

Lipid modulators may be purified from complex biological samples by use of a number of non-destructive methods (See Lipid Analysis in Oils and Fats, Edited by RJ Hamilton, Blackie Academic & Professional, 1997), such as exposure to iodine vapors, or exposure to a number of extraction solvents, such as chloroform-methanol (e.g. for extraction of cell membrane constituents), chloroform alone, ethanol-hexane, 2′7′-dichlorofluorescin (e.g.
a 0.2% solution of the compound in water), Rhodamine 6G, water (for purification of large amounts of lipids such as triglycerides), and lipids containing chromophores (e.g., for separation of triglycerides, methyl esters). Chromatographic methods (both gas and liquid chromatography) may also be used for separation of lipid modulators, alone or in combination with mass spectrometry.

**SYNTHESIS AND PRODUCTION OF MODULATORS**

Protein modulators may be synthesized via a number of methods that have been described in the art (See for example Graslund et al, *Nature Methods* 5, 135-146 (2008), including chemical synthesis methods, cell-free methods using prokaryotic or eukaryotic extracts, and genetically modified recombinant hosts such as prokaryotes (e.g., E. Coli), or eukaryotes (e.g., insect cells such as Baculovirus, yeasts such as Pichia Pastoris or Saccharomyces cerevisiae, or human cells).

Carbohydrate modulators may obtained by purification from an animal host (as detailed above) or chemically synthesized. In one embodiment, carbohydrate modulators are synthesized by a process that generally involves four parts, comprising preparation of glycosyl donors, preparation of glycosyl acceptors with a single unprotected hydroxyl group, coupling of these two groups, and a deprotection step (See for example John McMurry.; Organic Chemistry, 5th ed.; Brooks/Cole.; 2000, pp 1031).

Lipid modulators may be synthesized by biological or chemical methods described in the art (See for example "Fatty Acid and Lipid Chemistry", Edited by E. Gunstone. Blackie Academic. &. Professional, 1996). For example, chemical methods may comprise synthesis via acetylenic intermediates or via the Wittig reaction.

Sources for any materials may be of animal, plant, or synthetic origin. In one embodiment, materials may be isolated using methods known in the art and common in preparation of commercial food ingredients from commercial livestock.

**METHODS TO CREATE SYNTHETIC VARIANTS OF NATURAL MODULATORS WITH IMPROVED THERAPEUTIC PROPERTIES**

Natural modulators identified via the methods outlined above may be chemically modified and screened for enhanced therapeutic properties, such
as binding affinity to a target, pharmacokinetics, reactivity, stability to metabolic degradation, low toxicity, or selectivity for a target. Numerous methods to improve the properties have been described in the art, comprising both computer-aided design methods, rational drug design methods, random high throughput screening, and screening of focused libraries, among others (See for example "Medicinal chemistry: a molecular and biochemical approach" Thomas Nogrady and Donald F. Weaver; Oxford, 2005). The methods are generally used for the optimization of small molecule compounds, but can also be extended to optimize protein modulators (See, for example, Hartley et al, Proc. Nat. Acad. Sci. 101 (47), 2004), for example, via the introduction of unnatural amino acid analogs during the protein synthesis steps. Similarly, carbohydrate modulators can be optimized, for example, by using carbohydrate scaffolds to generate libraries via multicomponent reactions followed by screening (See, for example, Scheffler et al, J. Chem. Soc. Perkin Trans. 1, 2000)

**FORMULATIONS AND ADMINISTRATION**

Formulations are prepared using a pharmaceutically acceptable "carrier" composed of materials that are considered safe and effective and may be administered to an individual without causing undesirable biological side effects or unwanted interactions. The "carrier" is all components present in the pharmaceutical formulation other than the active ingredient or ingredients. The term "carrier" includes but is not limited to diluents, binders, lubricants, desintegrators, fillers, and coating compositions.

"Carrier" also includes all components of the coating composition which may include plasticizers, pigments, colorants, stabilizing agents, and glidants. Delayed release dosage formulations may be prepared as described in references such as "Pharmaceutical dosage form tablets", eds. Liberman et. al. (New York, Marcel Dekker, Inc., 1989), "Remington - The science and practice of pharmacy", 20th ed., Lippincott Williams & Wilkins, Baltimore, MD, 2000, and "Pharmaceutical dosage forms and drug delivery systems", 6th Edition, Ansel et.al., (Media, PA: Williams and Wilkins, 1995) which provides information on carriers, materials, equipment and process for preparing tablets and capsules and delayed release dosage forms of tablets, capsules, and granules.
As will be appreciated by those skilled in the art and as described in the pertinent texts and literature, a number of methods are available for preparing drug-containing tablets, beads, granules or particles that provide a variety of drug release profiles. Such methods include, but are not limited to, the following: coating a drug or drug-containing composition with an appropriate coating material, typically although not necessarily incorporating a polymeric material, increasing drug particle size, placing the drug within a matrix, and forming complexes of the drug with a suitable complexing agent.

Formulations intended for modulation of microbes present in mucosal surfaces may further comprise mucoadhesive components such as adhesive polymers. In general terms, adhesion of polymers to tissues may be achieved by (i) physical or mechanical bonds, (ii) primary or covalent chemical bonds, and/or (iii) secondary chemical bonds (i.e., ionic). Physical or mechanical bonds can result from deposition and inclusion of the adhesive material in the crevices of the mucus or the folds of the mucosa. Secondary chemical bonds, contributing to bioadhesive properties, consist of dispersive interactions (i.e., Van der Waals interactions) and stronger specific interactions, which include hydrogen bonds. The hydrophilic functional groups responsible for forming hydrogen bonds are the hydroxyl (-OH) and the carboxylic groups (-COOH). Suitable polymers that can be used to form bioadhesive particles include soluble and insoluble, biodegradable and nonbiodegradable polymers. These can be hydrogels or thermoplastics, homopolymers, copolymers or blends, natural or synthetic.

Formulations may further comprise natural polymers including proteins, such as zein, modified zein, casein, gelatin, gluten, serum albumin, or collagen, and polysaccharides, such as cellulose, dextrans, polyhyaluronic acid, polymers of acrylic and methacrylic esters and alginic acid.

Formulations may be designed for optimized delivery to the colon. Numerous methods have been described in the art to enable general delivery of drugs to the colon. Such methods include pH-sensitive formulations (e.g. formulations coated with enteric polymers that release drug when the pH move towards a more alkaline range, after passage through the stomach), formulations that delay the release of the drug for a lag time of 3-5 hours, roughly equivalent to small intestinal transit time, thereby securing delivery...
to the colon, drugs coated with bioadhesive polymers that selectively provide adhesion to the colonic mucosa (e.g. see U.S. Patent No. 6,368,586), and delivery systems that incorporate protease inhibitors to prevent proteolytic activity in the gastrointestinal tract from degrading biologic drug agents.

Formulations may also be designed for vaginal, skin, ear, and airway delivery. Modulators targeting bacterial niches in the vagina may be ideally delivered by administration in the form of suppositories. Modulators targeting bacterial niches in the skin may be ideally delivered by formulation into creams, gels, lotions, skin patches, and skin microneedle systems, all of which have been extensively described in the art. Modulators targeting bacterial niches in the nasal cavity may be ideally delivered by formulation into aerosols. Modulators targeting bacterial niches in the ear may be ideally delivered by formulation into drops. Modulators targeting bacterial niches in the airways and lung may be ideally delivered by formulation into aerosols. Aerosols for the delivery of therapeutic agents to the respiratory tract have been described, for example, Adjei, A. and Garren, J. Pharm. Res., 7: 565-569 (1990); and Zanen, P. and Lamm, J.-W.J. Int. J. Pharm., 114: 111-115 (1995).

**Combinations**

The compositions described herein may also be combined with known microbiota modulators to create more effective or specific compositions. These known modulators can include prebiotics, probiotics, or antibiotics.

Most known prebiotics are usually plant-derived complex carbohydrates or polysaccharides, although they may be non-carbohydrate and/or of non-plant origin. Generally, these materials are undigestible or poorly digested by humans, and thus remain intact through the proximal distal tract until the colon, where they serve as a food source for colonic bacteria. Prebiotics allow for the growth of specific sets of bacteria, and/or decrease or inhibit the growth of other sets of bacteria. Additionally, prebiotics may preferentially cause microbes to "turn on" biochemical pathways beneficial to health, or "turn off" biochemical pathways detrimental to health (such as toxin producing pathways). Prebiotics which can be used in the combination therapies include, without limitation,
galactooligosaccharides (GOS), trans-galactooligosaccharides, fructooligosaccharides or oligofructose (FOS), inulin, oligofructose-enriched inulin, lactulose, arabinofuranose, xylo-oligosaccharides, manno-oligosaccharides, and combinations of thereof.

Another approach for modulating the microbes present in a subject is using one or more probiotics. Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Common types of probiotics include lactic acid bacteria (LAB) and Bifidobacteria, although some yeast strains and bacillus strains have also been used. These microorganisms are often naturally found in fermented products like yogurt, kefir, buttermilk and pickled vegetables, or are added to dietary supplements and foods alike. Probiotics have also been used in topical formulations such as creams, and in formulations for administration to the mouth and vagina. Indications in which probiotics have previously been used include the treatment of lactose intolerance, antibiotic-associated diarrhea, inflammatory bowel disease, Crohn's disease and gastrointestinal enteric infections. Probiotics elicit these beneficial effects through, for example, the competitive exclusion and the displacement of certain unfavorable microorganisms from a specific ecological niche. Therefore probiotics can be used to alter the compositions and functionalities of microbiomes. Known probiotics include strains selected, individually or in combination, and include those from the Lactobacillus spp., Bifidobacteria spp., Saccharomyces spp., Bacillus spp., Lactococcus spp., Firmicutes, Actinobacteria, Acinetobacter spp., Bacillales, and Bacteroidetes. These microbial metabolism modulators have the capacity of shifting the colonic microflora composition or activity, and can be employed to alter a microbial dysbiosis to provide metabolic and physiological benefits to the host.

Various symbiotic combinations include combinations of prebiotics and probiotics called synbiotics. In one embodiment, the synbiotic combines a prebiotic and probiotic that either alone or together have demonstrated beneficial effects. Additionally, a synbiotic can be beneficial if even one component has demonstrated an effect of interest and the other component serves a presumed effect, or a general health, maintenance, or structural effect. Methods for modulating the composition of the gut microbiome
entail selecting and dosing suitable prebiotics and probiotics, or combinations of thereof, known by those skilled in the art synbiotics.

Still another approach for modulating the production of specific microbial metabolites is to alter the distribution of microbes present in a subject using antibiotics and bacteriocins. Antibiotics which can be used in the combination therapies include, without limitation, amoxicillin/clavulanate, aminoglycosides (oral) other than tobramycin, ampicillin/sulbactam, amphotomycin ristocetin, azithromycin, bacitracin, buforin II, carbomycin, cephalosporins (oral), cecropin PI, clarithromycin, erythromycins, furazolidone, other nitrofurans, fusidic acid, Na fusidate, gramicidin, glycopeptides, imipenem (oral), other penems, indolicidin, josamycin, linezolid, other oxazolidinones, magainan II, macroolides, metronidazole, other nitroimidazoles, mikamycin, mutacin B-Ny266, mutacin B-JH1 140, mutacin J-T8, nisin, nisin A, novobiocin, oleandomycin, ostreogrycin, piperaclillin/tazobactam, pristinamycin, ramoplanin, ranalexin, reuterin, rifaximin, rosamicin, rosaramicin, spectinomycin, spiramycin, staphylomycin, streptogramin, streptogramin A, synergistin, taurolidine, teicoplanin, telithromycin, ticarcillin/clavulanic acid, triacylloleandomycin, tylosin, tyrocidin, tyrothricin, vancomycin, vemamycin, and virginiamycin. In certain embodiments, the combination therapies include an antibiotic such as metronidazole, linezolid, fidaxomicin, rapplamacin, cholestyramine, nitaoxanide, rifaximin, diflimicin, and vancomycin. Exemplary bacteriocins which can be used in the combination therapies include, without limitation, Thuricin CD, Nicin, Thermophilin 13, Brochocin A/B/C, Circularin A, Bovicin 255, Closticin 57 4, Gassericin, Enterocin L50, and Enrocin EJ97 (MeIo Coutinho HD et al, Indian Journal of Pharmacology, 40:3(2008)).

**EXAMPLES**

The present invention will be further understood by reference to the following hypothetical examples.

Any of the below examples may also include a probiotic, known prebiotic, or antibiotic specifically selected to modulate the microbiome in the specific niche towards the healthy state.
EXAMPLE 1. Mouthwash

An oral rinse, to be washed in the mouth but not swallowed, directed for the treatment of bad breath and general promotion of beneficial bacteria, each approximately 30 mL dose including:

<table>
<thead>
<tr>
<th>Component</th>
<th>amounts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium phosphate (salivary electrolyte)</td>
<td>between 0.5 and 10 mg/mL</td>
</tr>
<tr>
<td>Calcium citrate (salivary electrolyte)</td>
<td>between 0.1 and 5 mg/mL</td>
</tr>
<tr>
<td>MUC5B (salivary mucin)</td>
<td>between 10 and 100 ug/mL</td>
</tr>
<tr>
<td>Urea (salivary molecule)</td>
<td>between 0.1 and 2 mg/mL</td>
</tr>
<tr>
<td>Maltose (salivary digestive product)</td>
<td>between 1 and 10 mg/mL</td>
</tr>
<tr>
<td>Inactive ingredients (glycerin, saccharin, sodium benzoate, acceptable colorings)</td>
<td>total 1 mg</td>
</tr>
<tr>
<td>Water</td>
<td>0.8-0.99 mL/total mL</td>
</tr>
</tbody>
</table>

EXAMPLE 2. Mouthwash

An oral rinse, to be washed in the mouth but not swallowed, directed for the treatment of bad breath and general promotion of beneficial bacteria, each dose including:

<table>
<thead>
<tr>
<th>Component</th>
<th>weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium phosphate (salivary electrolyte)</td>
<td>0.5%</td>
</tr>
<tr>
<td>Calcium citrate (salivary electrolyte)</td>
<td>0.2%</td>
</tr>
<tr>
<td>MUC5B (salivary mucin)</td>
<td>0.05%</td>
</tr>
<tr>
<td>Urea (salivary molecule)</td>
<td>0.1%</td>
</tr>
<tr>
<td>Maltose (salivary digestive product)</td>
<td>0.5%</td>
</tr>
<tr>
<td>Inactive ingredients (glycerin, saccharin, sodium benzoate, acceptable colorings)</td>
<td>0.5%</td>
</tr>
<tr>
<td>Water</td>
<td>98%</td>
</tr>
</tbody>
</table>
The above composition can alternately be employed in a solid matrix (preferably toothpaste, dental floss, or a dissolvable tab), with the relative percentage weights in the solubalized fraction equal to that above.

The above examples can optionally additionally include the following ingredients:
- salivary electrolyte cations (matched with a preferred anion) selected from potassium, calcium, magnesium
- salivary electrolyte anions (matched with a preferred cation) selected from chloride, bicarbonate, fluoride
- salivary sugars selected from glucose, dextrin, glycosaminoglycans
- salivary enzymes selected from amylase, lysozyme, peroxidase
- salivary antimicrobial peptides

**EXAMPLE 3: Skin Cream**

A skin cream, gel, or salve prepared and packaged for once-, twice-, or as needed administration to affected areas of the skin, to be used by a patient to prevent or treat a condition of microbial dysbiosis in the skin including eczema. The cream includes:

<table>
<thead>
<tr>
<th>Component</th>
<th>Wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbiota modulator (e.g., Keratin)</td>
<td>1-30%</td>
</tr>
<tr>
<td>Oil (e.g., olive oil, palm oil)</td>
<td>0-33%</td>
</tr>
<tr>
<td>Emulsifier (e.g., lecithin)</td>
<td>0-5%</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>0-10%</td>
</tr>
<tr>
<td>Alpha-hydroxy acid (skin softener)</td>
<td>0-5%</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>0-3%</td>
</tr>
<tr>
<td>Colorants and odorants</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Vitamins (e.g., vitamin C, E)</td>
<td>0-2%</td>
</tr>
<tr>
<td>Water</td>
<td>to 100% (usually 33-66%)</td>
</tr>
</tbody>
</table>

**EXAMPLE 4: Food Bar for Treatment of Microbial Dysbiosis:**

A foodstuff packaged in a preformulated 25 g food bar, to be consumed once-daily by patients to prevent or treat microbial dysbiosis including diarrhea or constipation.
The composition may additionally comprise mucus, hormones, water, inorganic salts (alkaline salts such as sodium bicarbonate), and active enzymes including proteases, amylases, lipases, peptidases, sucrases, maltases, enterokinases, alkaline phosphatases, nucleophosphatases, and nucleocytases.

**EXAMPLE 5: Aerosol for Treatment of Microbial Dysbiosis**

An aerosol formulated for once- or twice-daily pump spray administration to the nasopharynx, to be administered into the nose for the prevention or treatment of microbial dysbiosis causing or increasing susceptibility to infection, irritation, or allergy.

<table>
<thead>
<tr>
<th>Component</th>
<th>Wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complement factors (e.g., factors 3, 4)</td>
<td>0-1%</td>
</tr>
<tr>
<td>Mucins</td>
<td>0-5%</td>
</tr>
<tr>
<td>Carbohydrates (e.g., mucins, microcrystalline cellulose, dextrose)</td>
<td>0-5%</td>
</tr>
<tr>
<td>Buffering agents (e.g., hypochloric acid, sodium phosphate, sodium hydroxide) to a physiological pH</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Preservative (e.g., disodium EDTA)</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Purified water</td>
<td>to 100%</td>
</tr>
</tbody>
</table>

The composition may also optionally additionally comprise:

- Proteins including alpha 1-antitrypsin, albumin, hemoglobin, apolipoprotein
- Immunoglobulins such as IgA
- Carbohydrates such as common constituents of mucin such as galactose, fucose, mannose, galactose, sialic acids, uronic acids chondrosamine, and glucosamine, among others.

**EXAMPLE 6. Vaginal Wash**

A vaginal wash, to be administered to the vagina for routine health, during symptoms of an infection, or before or after intercourse:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amounts</th>
</tr>
</thead>
<tbody>
<tr>
<td>S100A8/9 (vaginal proteins)</td>
<td>between 0.1 and 1 mg/mL</td>
</tr>
<tr>
<td></td>
<td>(or 0.1% by weight)</td>
</tr>
<tr>
<td>Mucin (vaginal mucous component)</td>
<td>between 0.2 and 1 mg/mL</td>
</tr>
<tr>
<td></td>
<td>(0.1%)</td>
</tr>
<tr>
<td>Cathelicidin (vaginal antimicrobial molecule)</td>
<td>between 10 and 100 ug/mL</td>
</tr>
<tr>
<td></td>
<td>(0.05%)</td>
</tr>
<tr>
<td>Beta-defensin (vaginal antimicrobial molecule)</td>
<td>between 10 and 100 ug/mL</td>
</tr>
<tr>
<td></td>
<td>(0.05%)</td>
</tr>
<tr>
<td>Lactic acid/ sodium lactate (vaginal microbe</td>
<td>variable amount</td>
</tr>
<tr>
<td>dependent on secretion and pH buffer 3.8)</td>
<td></td>
</tr>
<tr>
<td>Specific buffering need</td>
<td></td>
</tr>
<tr>
<td>Benzoic acid/ Sodium benzoate (pH buffer 4.2)</td>
<td>variable amount</td>
</tr>
<tr>
<td>dependent on specific buffering need</td>
<td></td>
</tr>
<tr>
<td>Inactive ingredients (glycerol, vitamins,</td>
<td>up to 500 mg/mL</td>
</tr>
<tr>
<td>acceptable excipients and aromas)</td>
<td>(30%)</td>
</tr>
<tr>
<td>Water</td>
<td>50-99%</td>
</tr>
</tbody>
</table>

**EXAMPLE 7. Vaginal Cream or Suppository**

A health-promoting vaginal cream or suppository, with substantially similar composition as the example above, additionally comprising:

- propylene glycol (viscositizing agent) - up to 20%
- calcium alginate (viscositizing agent and prebiotic) - up to 2%
- guar gum (viscositizing agent and prebiotic) - up to 2%
We claim:

1. A method for identifying microbiota modulators comprising:
   (i) selecting an animal microbial niche in or on an organ;
   (ii) identifying one or more natural compositions in the niche;
   (iii) purifying or producing recombinantly or synthetically the one or more compositions;
   (iii) testing the compositions for microbiota modulating activity; and
   (iv) selecting a composition with beneficial microbiota modulating activity.

2. The method of claim 1 where the organ is an organ selected from the group consisting of skin, mouth, vagina, nose, or lungs.

3. The method of claim 1 where the organ is not the gut.

4. The method of any of claims 1-3 where the natural compositions are host compositions.

5. The method of any of claims 1-3 where the natural compositions are microbial compositions.

6. The method of claim 4 where the host compositions are constitutively present or produced.

7. The method of claim 4 where the host compositions are a significant fraction of the host tissue in the niche

8. The method of claim 4 where the host compositions are 1-100% of the wet mass of the tissue.

9. The method of claim 4 where the host compositions are 0.1-1% of the wet mass of the tissue.

10. The method of claim 4 where the host compositions are 0.01-0.1% of the wet mass of the tissue.
11. The method of any of claims 1-10 where the natural composition is metabolized by the modulated members of the microbiota as an energy source.

12. The method of any of claims 1-10 where the natural composition is toxic to members of the microbiota.

13. The method of any of claims 1-10 where the natural composition binds to a microbial target.

14. The method of any of claims 1-10 where the natural composition binds to a host target.

15. A microbiota modulator for the improvement of organ health comprising isolated natural compositions derived from that organ and selected for organ health.

16. A microbiota modulator for the improvement of health comprising isolated compositions present in the microbial niche during a healthy state and selected for improving health.

17. The composition of any of claims 15 or 16 where the organ is an organ selected from the skin, mouth, vagina, nose, or lungs.

18. The composition of any of claims 15 or 16 where the organ is not the gut.

19. The composition of any of claims 15, 17, or 18, where the natural compositions are host compositions.

20. The composition of any of claims 15, 16, 17 or 18 where the natural compositions are microbial compositions.

21. The composition of claim 19 where the host compositions are constitutively present or produced.

22. The composition of claim 19 where the host compositions are a significant fraction of the host tissue in the niche.
23. The composition of claim 22 where the host compositions are 1-100% of the wet mass of the tissue.

24. The composition of claim 22 where the host compositions are 0.1-1% of the wet mass of the tissue.

25. The composition of claim 22 where the host compositions are 0.01-0.1% of the wet mass of the tissue.

26. The composition of any of claims 15 or 16 where the natural composition is able to be metabolized by the modulated members of the microbiota as an energy source.

27. The composition of any of claims 15 or 16 where the natural composition is toxic to members of the microbiota.

28. The composition of any of claims 15 or 16 where the natural composition binds to a microbial target.

29. The composition of any of claims 15 or 16 where the natural composition binds to a host target.

30. The composition of any of claims 15 or 16 where the modulators are purified, recombinantly produced, or synthetically produced natural host compositions.

31. The composition of any of claims 15 or 16 where the modulators are chemically modified natural host compositions.

32. The composition of any of claims 15 or 16 where the modulators are synthetic mimics of natural host compositions.